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A SEM-BASED STUDY OF THE GYNOECIUM IN FRITILLARIA L. (LILIACEAE)

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Abstract
The systematic value of stigma, style, and ovary morphology in the subgeneric classification of *Fritillaria* (Liliaceae) was investigated using scanning electron microscopy (SEM) on 29 taxa. A quantitative and qualitative analysis of gynoecium morphology was conducted across the subgenera *Fritillaria*, *Rhinopetalum*, and *Petilium*. The research methodology involved quantitative measurements of stigma, style, and ovary lengths, complemented by qualitative evaluations of style ornamentation, style apex, style morphology, and ovary morphology. A clustering dendrogram was constructed based on these characters, and a phylogenetic tree was built using a phylogenetic backbone based on the botanical nomenclature of The Plant List in R software and Bray–Curtis dissimilarity. The results showed some correlation between the dendrogram and the phylogenetic tree, indicating the relative usefulness of a clustering dendrogram constructed based on the combined quantitative and qualitative characters. Moreover, phylogenetic analysis of the species was performed using the vascular plant mega-tree and the R package 'phylomaker' to construct a phylogenetic tree. A reference phylogenetic tree was used to evaluate the taxonomic accuracy of the dendrogram, with an assessment based on gynoecium characters. The gynoecium characters within *Fritillaria* were useful in a few subgenera and had limited significance in the subgeneric classification of the genus.

Keywords: *Fritillaria*; gynoecium; Liliaceae; ovary; SEM; stigma; style

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مطالعه مادگی گل در جنس لاله سرنگون (Fritillaria)، (Liliaceae) با استفاده از میکروسکوپ الکترونی

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چکیده: ارزش سیستماتیک مورفولوژی کلاله، خامه و تخمدان در طبقه‌بندی لاله سرنگون (*Fritillaria* L.-Liliaceae) با استفاده از میکروسکوپ الکترونی روبشی (SEM) بر روی ۲۹ آرایه مورد بررسی قرار گرفت. تجزیه‌وتحلیل کمی و کیفی مورفولوژی مادگی در همه زیرجنس‌های *Fritillaria*، *Rhinopetalum* و *Petilium* انجام شد. روش تحقیق شامل اندازه‌گیری‌های کمی کلاله، خامه و طول تخمدان بود که با ارزیابی‌های کیفی تزئینات کلاله، راس کلاله، مورفولوژی کلاله و مورفولوژی تخمدان تکمیل شد. یک دندروگرام خوشه‌بندی بر اساس این صفات ساخته شد، و یک درخت فیلوژنتیک با استفاده

از یک ستون فقرات فیلوژنتیکی بر اساس اصول نامگذاری بانک اطلاعات لیست گیاهان (The Plant List) در نرم‌افزار R و عدم تشابه Bray-Curtis ساخته شد. نتایج نشان داد که بین دندروگرام و درخت فیلوژنتیک همبستگی وجود دارد که نشان‌دهنده سودمندی نسبی دندروگرام خوشه‌بندی ساخته شده براساس صفات ترکیبی کمی و کیفی است. علاوه بر این، تجزیه و تحلیل فیلوژنتیک گونه‌ها با استفاده از مگا درخت گیاه آوندی و بسته 'phylomaker R' برای ساخت یک درخت فیلوژنتیک انجام شد. یک درخت فیلوژنتیک مرجع برای ارزیابی دقت طبقه‌بندی دندروگرام، با ارزیابی بر اساس صفات مادگی استفاده شد. مقایسه گروه‌بندی‌های حاصل از آنالیز صفات مورفولوژیکی با گروه‌های حاصل از آنالیز فیلوژنتیکی نشان داد که تشابهی بین این گروه‌بندی‌ها وجود ندارد و خصوصیات مورفولوژیکی مادگی برای طبقه‌بندی زیرجنس‌ها نمی‌تواند مورد استفاده قرار گیرند.

INTRODUCTION

The genus *Fritillaria* L. (Liliaceae), encompassing approximately 140 geophytic species (Rix & al. 2001), presents considerable taxonomic challenges. The geographic distribution of these plants is limited to the temperate zones of the Northern Hemisphere (Rønsted & al. 2005), encompassing Europe, East and West Asia, the Mediterranean region, and North America (Beetle 1944). Bakhshi Khaniki (2023) provided the most recent taxonomic revision of the genus *Fritillaria* in Iran for the Flora of Iran. The potential value of androecium morphology for subgeneric classification within the genus *Fritillaria* was evaluated and found to be significant (Mohsennezhad & al. 2024).

Subgeneric relationships within the genus *Fritillaria* have been the focus of prolonged scholarly disagreement. The Linnaean classification of 1753 has been followed by several taxonomic treatments of *Fritillaria* subgenera. The bulb morphology, style, nectaries (Khaniki & al. 1997), and capsule structure were used in taxonomic treatments. Baker's 1874 publication proposes a subgeneric classification of the genus, comprising ten subgenera. Boissier (1884) classified section *Fritillaria* into two subsections based on style morphology, specifically whether the style was entire or trifid. The subgeneric taxa *Korolkowia* and *Rhinopetalum*, as defined by Baker (1874), were recognized as separate genera by Komarov and Losina-Losinskaya (1935). The morphological analysis of floral nectaries conducted by Bakhshi Khaniki (1997) and (Khaniki & al., 1997) resulted in the reclassification of the *Rhinopetalum* group as a separate genus, a finding consistent with Komarov's (1935). Karyological analyses revealed a paucity of heterochromatin in Old World taxa, contrasting with the abundance observed in New World taxa (Bakhshi Khaniki 1998). The current taxonomic classification of *Fritillaria* is based on the work of Rix & al., encompassing eight subgenera: *Davidii*, *Liliorhiza*, *Japonica*, *Fritillaria*, *Rhinopetalum*, *Petilium*, *Theresia*, and *Korolkowia* (Rix 1977).

Phylogenetic investigations, utilizing various genomic and organellar DNA sequences, were undertaken to resolve taxonomic uncertainties within *Fritillaria* (Rønsted & al. 2005). The foundation for this research is comprised of DNA sequence analyses of the matK, trnK intron, rpl16 ribosomal gene intron, and nrDNA-ITS regions (Rønsted & al.2005). Phylogenetic analysis revealed two principal clades within the genus, one encompassing the subgenus *Liliorhiza.* The second group is composed of members from the remaining seven subgenera. Furthermore, phylogenetic analysis utilizing matK and rpl16 sequences did not corroborate the monophyly of *Fritillaria*. Monophyly of the currently recognized subgenera was supported by analysis of three plastid genome regions. The Japanese endemic subgenus *Japonica*, characterized by possessing the largest recorded genome among diploid plants, is identified as the sister group to the predominantly Middle Eastern and Central Asian subgenus *Rhinopetalum* (Day & al. 2014). Subgeneric systematic revisions within the genus were conducted using scanning electron microscopy of the filament and anther. A comprehensive analysis of 27 *Fritillaria* species was conducted, encompassing quantitative and qualitative characteristics including filament and anther morphology, dimensions, color, ornamentation, and point of attachment. A correspondence between the dendrogram and phylogenetic tree was observed in the results, implying the comparative value of filament and anther ultrastructural characteristics for *Fritillaria* subgeneric classification. Furthermore, both phylogenetic analyses consistently indicated that the subgenus *Fritillaria* is non-monophyletic with a sister relationship observed between subgenera *Petilium* and *Theresia* (Mohsennezhad & al. 2024). This research employs scanning electron microscopy (SEM) to analyze the gynoecium, providing detailed observations of its pubescence and style divisions. A literature review reveals a paucity of comparative studies incorporating these characters to date. The

study's objective was to survey gynoecium diversity in South West Asian *Fritillaria* and *Rhinopetalum*, and to determine if gynoecium features are correlated with recent taxonomic revisions of these genera and subgenera. The ovary exhibits tricarpellate and trilobular morphology. Taxonomists have emphasized the branching and structural aspects of the style. This organ's functionality usually provides highly valuable traits. This research investigates the systematic utility of gynoecial characters within *Fritillaria* subgenera using scanning electron microscopy.

MATERIAL AND METHODS

Taxon sampling

Twenty-eight taxa, representing three of the ten recognized subgenera within *Fritillaria*, *Rhinopetalum* Fisch., and *Petilium* Endl. from the genus *Fritillaria* were included in the study. Plant specimens were sourced by the authors from Iran and/or cultivated in collections at the Gothenburg Botanical Garden (GB), Sweden. Vouchers are archived within the Herbarium at Gothenburg University, Sweden. Each population in the following is assigned a collection number preceded by the abbreviation Gholamreza Bakhshi Khaniki (GBK) which stands for author name (Table 1). Taxonomic consistency was maintained by consulting The Plant List (http://www.theplantlist.org) and the Royal Botanic Gardens, Kew's Plants of the World Online (https://powo.science.kew.org/). A minimum of four samples from each species were randomly chosen, and the average was utilized in the subsequent analysis.

Morphological studies

The shape and size of the ovary and the division and pubescence of styles were studied on fresh material by light microscope. Styles were also fixed in FAA (5 vol. Formalin 40%; 5 vol. Acetic acid, glacial; 90 vol. Ethanol 50%) in the greenhouse, and preparations were observed with a Carl Zeiss photo stereo microscope using Camera Lucida. Styles for SEM studies were prepared as follows: Mature and well-developed styles were separated from the flower and transferred to 70% alcohol for 2 days. After this, the material was kept in 70% acetone and then dehydrated in a graded acetone series (90% and 95%; 15 min each) and further dehydrated twice in 100% acetone (20 min each). The material was subjected to critical point drying (BALZERS CPD 030) with liquid CO2 as a transitional fluid and thereafter coated with gold (50 nm thick, BALZERS UNION 010). The material was studied using a Zeiss scanning electron microscope (SEM, ZEISS DSM 940).

Analysis

The gynoecium morphology of 24 *Fritillaria* taxa was characterized using three quantitative variables (stigma, style, and ovary length) and four qualitative variables (styles ornamentation, style apex shape, styles shape, ovary shape), as detailed in Tables 2,3 and 4. The average values of morphological characters were used in the analysis. Taxa possessing fewer than four measurable characters were omitted from the analysis due to limitations in data acquisition, resulting in the inclusion of 26 taxa in the study. Morphological quantitative characteristics of the gynoecium in *Fritillaria* species are shown in Table 3; the qualitative characteristics of the gynoecium are shown in Tables 4 and 5. A clustering dendrogram was constructed based on quantitative and qualitative stigma, style, and ovary variables, using Principal Component Analysis in R software 4.3.2 (R Core Team 2023) and Bray–Curtis dissimilarity. A comparison of the gynoecium-based dendrogram with the phylogenetic tree to evaluate the systematic utility of gynoecium characteristics (Rønsted et al. 2005, Day & al. 2014). Both the dendrogram and the phylogenetic tree are shown in. Fig. 13.

RESULTS

The species under study are illustrated via scanning electron microscope (SEM) micrographs in Figs. 1-12. Significant variation in style morphology was observed across *Fritillaria* subgenera *Petilium*, *Fritillaria*, *Theresia*, and *Rheinopetalum.* The qualitative characteristics were the main factor in grouping the species, but the quantitative characteristics were responsible for dividing the species into smaller groups. In the studied species style was entire or branched. The length of branches differed in different species groups. The pubescence of styles is also useful for subgeneric characterization and formal grouping. The styles in studied species are glabrous, densely papillose, or minutely papillose. The ovary is characterized by three carpels and three locules in all species. It is cylindrical, oblong, or obconical-cylindrical, 3.25-15 mm long (Tables 2,3, 5).

The results of the analyses of gynoecium characteristics provided a cladogram, clustering the species into three groups (I, II, III) (Fig. 13). The characteristics of the groups are presented in Table 2. The specific characteristics of groups and species belonging to each group are explained below:

Table 1. The source of *Fritillaria* materials used in the current study GBK= Gholamreza Bakhshi Khaniki.
*Plant materials collected from cultivated plants in Göteborg Botanical Garden.
Vouchers are deposited in the Herbarium of the Goteborg University GB Sweden. Each population in the following is assigned a collection number preceded by the abbreviation GBK which stands for the author.

No.	Taxa	Subgenera	Locality
1	*Fritillaria ariana* (Losinsk. & Vved.) Rix	*Rhinopetalum*	Iran: Khorasan, Torbat-e Jam, Salehabad 1500 m, GBK 42
2	*F. armena* Boiss. *	*Fritillaria*	Turkey: Erzurum, east of Kop pass, SØNDERHOUSEN 1134
3	*F. assyriaca* Baker*	*Fritillaria*	Turkey: Agri, Tahir Da. pass, SØNDERHOUSEN 1106
4	*F. atrolineata* Bakhshi Khan.	*Fritillaria*	Iran: W. Azarbaijan, Urmiah, Ghasemlu, 1500 m, GBK 63
5	*F. bucharica* Regel.	*Rhinopetalum*	Tadjikistan: Hissar mountains, 1400 m, VACRATOT
6	*F. caucasica* Adams	*Fritillaria*	Iran: E. Azarbaijan, Tabriz to Ahar, 1800 m, GBK 70
7	*F. chlorantha* Hausskn. & Bornm.	*Fritillaria*	Iran: Khorramabad, Oshtoran Kuh, 2400 m, GBK 88
8	*F. chlororhabdota* Bakhshi Khan.	*Fritillaria*	Iran: W. Azarbaijan, Urmiah, Sir mtns., 1800 m, GBK 65
9	*F. crassifolia* Boiss. & A. Huet*	*Fritillaria*	Turkey: Denizli, Honaz Dag, STEVENS
10	*F. crassifolia* subsp. *kurdica* (Boiss. & Noë) Rix	*Fritillaria*	Iran: Salmas, Ghooschi pass, 1900 m, GBK 67
11	*F. gibbosa* Boiss.	*Rhinopetalum*	Iran: Tehran, Karadje, Park-e Chitgar, 1400 m, GBK 1
12	*F. hermonis subsp. amana* Rix*	*Fritillaria*	Turkey: Hatay, 5 km before Belen, 1300 m, SØNDERHOUSEN 1055
13	*F. imperialis* L.	*Petilium*	Iran: Esfahan, Khunsar, Golestan Kuh, 2600 m, GBK 16
14	*F. karelinii* (Fisch. ex D.Don) Baker*	*Rhinopetalum*	Turkmenistan, STEVENS
15	*F. kotschyana* subsp. *grandiflora* (Grossh.) Rix*	*Fritillaria*	Turkmenistan: Talysh, Pagum Lerik, 1100 m, FURSE 3520
16	*F. michailovskyi* Fomin*	*Fritillaria*	Turkey: Kars, Sarikamis, Yenikoy, 2300 m, SØNDERHOUSEN 893
17	*F. minima* Rix*	*Fritillaria*	Turkey: Van, Kavussahap Dag, MATHEW
18	*F. minuta* Boiss. & Noë *	*Rhinopetalum*	Turkey: Van, Tatvan, 2510 m, FURSE 7241
19	*F. olivieri* Baker	*Fritillaria*	Iran: Hamadan, Kuh-e Elvand, 2500 m, GBK 60
20	*F. pinardii* Boiss.*	*Fritillaria*	Turkey: Eskisehir, 22 km north of Eskisehir, SØNDERHOUSEN 1219
21	*F. poluninii*(Rix) Bakhshi Khan. & K.M. Perss.*	*Fritillaria*	Iran: Kermanshahan, Kuh-e Owraman, pass between Daraki and Nowsud, 2500-2600 m, WENDELBO
22	*F. raddeana* Regel	*Petilium*	Iran: Gorgan, Golestan Forest, Almeh, 1650 m, GBK 48
23	*F. reuteri* Boiss.	*Fritillaria*	Iran: W. Azarbaijan, Urmiah, Kuh-e Sir, 2100 m, GBK 94
24	*F. stenanthera* (Regel) Regel *	*Rhinopetalum*	Turkmenistan: near Askabad, CUBA
25	*F. straussii* Bornm.	*Fritillaria*	Iran: Kermanshah, Ghallajeh pass, 1700 m, GBK 51
26	*F. uva-vulpis* Rix *	*Fritillaria*	Iraq: Rowandooz, Haji Omran, TUBERGEN
27	*F. whittallii* Baker *	*Fritillaria*	Turkey: Southwest Turkey, Mediterranean region central Anatolian Plateau, Taurus Mountains.
28	*F. zagrica* Stapf	*Fritillaria*	Iran: Arak, Soltanabad village, 1700-1900 m, GBK 33

Table 2. The characteristics of groups of *Fritillaria* species (Group I, Group II, Group III).

	Taxa.	Styles indumentum	Styles apex	Styles shape	Style length (mm)	Ovary length (mm)	Stigma length (mm)
Groupe I	*F. atrolineata*	Densely papillose	Entire or slightly 3-lobed	Stout	5-7 (6)	6-8 (7)	2.65
	F. poluninii	Minutely papillose toward apex	Trifid for 1/2 of its length	Slender to narrowly stout	4-5 (4.5)	3-4.5 (3.75)	2.25
	F. bucharicua	Glabrous	Entire	Slender	3-4	4	1.5
	F. chlororhabdota	Densely papillose	Entire	Stout	6-8	8-10	3.65
	F. zagrica	Papillose	Entire	Slender	7-9	4-5	1.75
	F. karelinii	Papillose only at apex (stigma)	Entire	Slender	8-11	5-8	1.75
	F. stenanthera	Densely papillose	Entire	Slender	5-7	4-5	1.4
	F. minuta	Glabrous	Trifid, 2.5-4 mm long	Stout	4-7.3	3-4	3.25
	F. arianua	Glabrous	Entire	Slender	5-7	4-5.5	1
	F. gibbosa	Glabrous	Entire	Slender	6-13	5-6	1
Groupe II	*F. caucasica*	Papillose	Trifid for 1-2.5 mm long	Slender	9-16	5-6	1.75
	F. chlorantha	Papillose	Trifid for 1-2.5 mm long	Stout	6-8	3-4	1.25
	F. kotschyana ssp. *grandiflora*	Minutely papillose toward apex	Trifid for 2.5-4 mm long	Narrowly obconical stout and entire	8	4.5	2.75
	F. reuteri	Papillose	Trifid for 2.5-4 mm long	Slender to narrowly stout	7-13	6	3
	F. minima	Papillose	trifid for 1-2.5 mm long	Slender to narrowly stout	5-8	2.5-4	1.75
	F. imperialis	Papillose	trifid for 1-2.5 mm long	Stout	26-45	14-16	3.25
	F. raddeana	Papillose	trifid for 1-2.5 mm long	Slender	18-27	5	1.75
Groupe III	*F. michailovskyi*	Papillose	trifid early to the middle with the branches 2-3.5 mm	Slender to narrowly stout	7-9	5-7	2.75
	F. assyriaca	Densely papillose	trifid for 1-2.5 mm long	Stout	6-8	6-7	1.5
	F. straussii	Papillose	trifid almost from the base	Stout	6-10	6-7	4
	F. crassifolia ssp. *kurdica*	Glabrous	trifid for 1/4 to 1/5 of its length with branches 1-2 mm	Slender to narrowly stout	5-9	5-6	1,5
	F. olivieri,	Glabrous	trifid for 1/3 to 1/4 of the length	Narrowly obconical stout and entire	6.5-9	8-10	2.25
	F. hermonis ssp. *amana*	Papillose	trifid almost from the base	Narrowly obconical stout and entire	8-12	6-7.5	2.25
	F. pinardii	Densely papillose	Entire	Stout	7-10	4-5.5	2.65
	F. uva-vulpis	Densely papillose	Entire	Narrowly obconical stout and entire	4-7	4-6	2.25
	F. whittallii	Glabrous	trifid for 3-4.5 mm long	Slender	9-11.5	8	3.75

Table 3: Morphological quantitative characters of gynoecium in *Fritillaria* species.

N0.	Taxa	Stigma length (mm)	Style length (mm)	Ovary length (mm)
1	*F. ariana*	1	6	4.75
2	*F. armena*	1.25	8.5	4.5
3	*F. assyriaca*	1.5	7	6.5
4	*F. atrolineata*	2.65	6	7
5	*F. bucharica*	1.5	3.5	4
6	*F. caucasica*	1.75	12.5	5.5
7	*F. chlorantha*	1.25	7	3.5
8	*F. chlororhabdota*	3.65	7	9
9	*F. crassifolia* ssp. *Crassifolia*	4.25	8.5	8
10	*F. crassifolia* ssp. *kurdica*	1,5	7	5,5
11	*F. gibbosa*	1	9.5	5.5
12	*F. hermonis*	2.25	10	6.75
13	*F. imperialis*	3.25	35.5	15
14	*F. karelinii*	1.75	9.5	6.5
15	*F. kotschyana grandiflora*	2.75	8	4.5
16	*F. michailovskyi*	2.75	8	6
17	*F. minima*	1.75	6.5	3.25
18	*F. minuta*	3.25	5.65	3.5
19	*F. olivieri*	2.25	7.75	9
20	*F. pinardii*	2.65	8.5	4.75
21	*F. poluninii*	2.25	4.5	3.75
22	*F. raddeana*	1.75	22.5	5
23	*F. reuteri*	3	10	6
24	*F. stenanthera*	1.4	6	4.5
25	*F. straussii*	4	8	6.5
26	*F. uva-vulpis*	2.25	5.5	5
27	*F. whittallii*	3.75	10.25	8
28	*F. zagrica*	1.75	8	4.5

Group I: Included *F. atrolineata*, *F. polunini*, *F. chlororhabdota*, *F. zagrica*, *F. minuta* of the subgenus *Fritillaria*, and *F. bucharica F. karelinii*, *F. stenantherum F. ariana*, and *F. gibbosa* of the subgenus *Rhinopetalum*. The species within this group have an entire or subentire style. Three subgroups shape this group, including subgroup a (*F. bucharica*, with glabrous styles and *F. chlorohabdota* with densely papillose styles), subgroup b (*F. zagrica, F. karelinii, F. stenantera*), slender and papillose styles, and subgroup c (*F. minuta, F. ariana, F. gibbosa*), with glabrous styles.

It should be noted that *F. atrolineata* with entre to slightly three fid, and densely papillose styles and *F. poluninii* with partly three fid and minutely papillose styles reside in independent branches.

Group II: Included *F. caucasica*, *F. chlorantha*, *F. kotschyana ssp. grandiflora*, *F. reuteri*, *F. minima*, of the subgenus *Fritillaria*, and *F. imperialis* and *F. raddeana* from subgenus *Petilium*. These species have papilose and 3-fid styles.

Three subgroups shape this group, including subgroup d (*F. caucasica*, *F. chlorantha*) with papilose, 3-fid styles, e (*F. kotschyana* ssp. *grandiflora*, *F.*

reuteri) with papilose, 3-fid styles, f (*F. minima, F. imperialis* and *F. raddeana*) with papilose, 3-fid styles. **Group III**: Included *F. michailovskyi, F. assyriaca, F. straussii, F. crassifolia ssp. kurdica, F. olivieri, F. hermonis* ssp. *amana, F. pinardii, F. uva-vulpis, F. whittallii* of the subgenus *Fritillaria*. Within this group, styles are 3-fid either from the base or from different parts of their lengths. There is an exception in this group, the *F. pinardii* and *F. uva-vulpis*, that have entire styles but share the papillose indumentum.

Three subgroups shape this group, including subgroup g (*F. michailovskyi, F. assyriaca, F. straussii*), with papillose and 3-fid styles, h (*F. crassifolia* ssp. *kurdica, F. olivieri*) with glabrous and 3-fid styles, and i (*F. hermonis* ssp. *Amana, F. pinardi*), with papillose styles, and (*F. uva-vulpis*, with densely papillose styles and *F. whittallii*, with glabrous styles).

DISCUSSION

Subgeneric classification of *Fritillaria* based on the gynoecium represents a specialized area of botanical study. In this study, we evaluated the implication of gynoecium characteristics in the subgeneric classification of *Fritillaria*. The qualitative characteristics were significant for grouping the related species. Studies on gynoecium morphology included stigma shape, style shape, size, indumentum, ovary structure, and other morphological traits that helped distinguish subgenera. The gynoecium morphology was incorporated in some previous subgeneric classifications. The stylar branching was one of the diagnostic characteristics used by Boissier (1884), who divided section *Fritillaria* into two subsections, characterized by entire and trifid styles. Our analysis shows that the style branching was responsible for grouping the studied species into three groups, but there were some exceptions in group III. The species in this group had mostly branched styles, but there were two entire style species (*F. pinardii* & F. *uva-vulpi*). Furthermore, variations in interspecific stylistic morphology including glabrous and papillose, slender or stout forms are responsible for further groupings as defined by Rix (1977). The analysis of style branching reveals subgeneric and sectional characters and further distinguishes closely related species through stylar characteristics (Bakhshi Khaniki, 1997 & 2023).

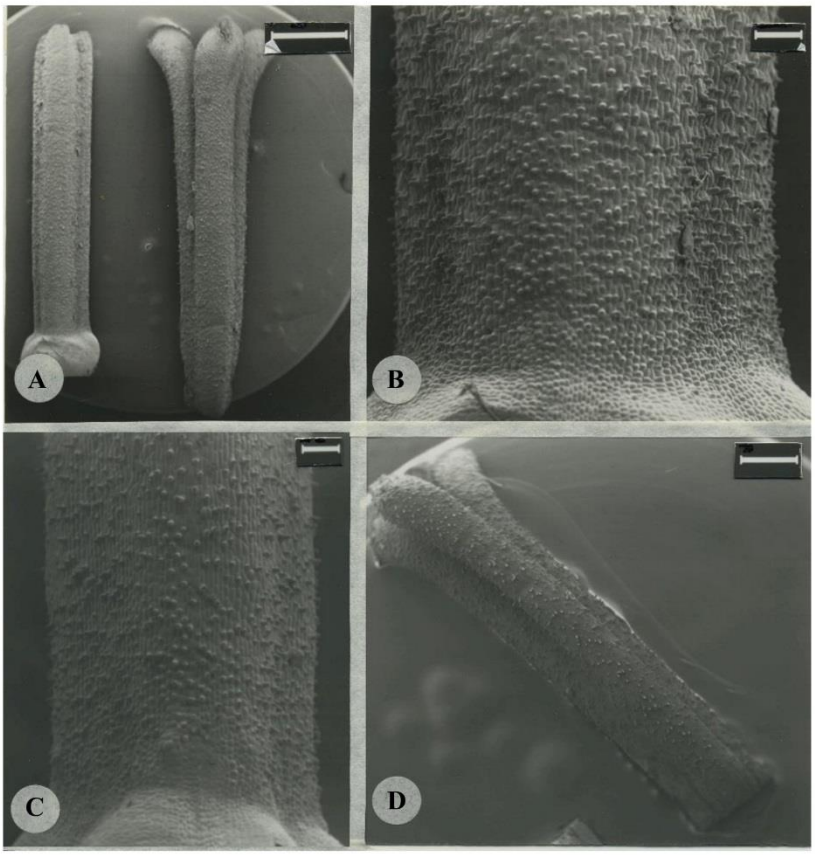

Fig. 1. SEM micrographs of papillose branched styles in *Fritillaria imperialis*: A, style; B, style base. Papillose branced styles in *Fritillaria raddeana*: C, style base; D, style. Scales: A= 2 mm; B= 50 µm; C= 200 µm; D= 1 mm.

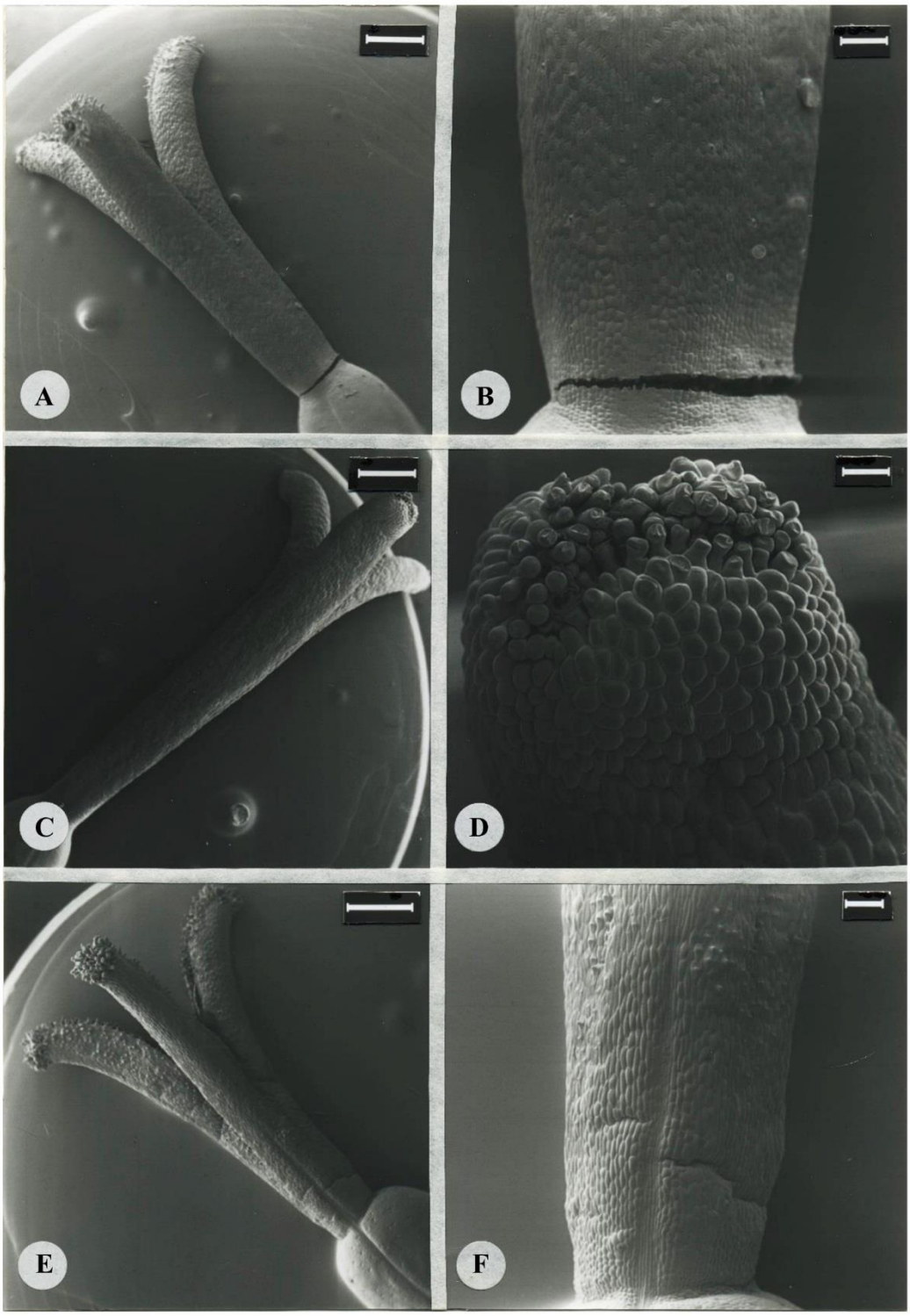

Fig. 2. SEM micrographs of glabrous type styles in *Fritillaria crassifolia* ssp. crassifolia: A, style; B, style base. Glabrous branched type styles in *Fritillaria crassifolia* ssp. kurdica: C, style; D, stigma surface. Minutely papillose branched type styles in *Fritillaria poluninii*: E, style; F, style base. Scales: A, C, E=1 mm; B & F=200 µm; D=100 µm.

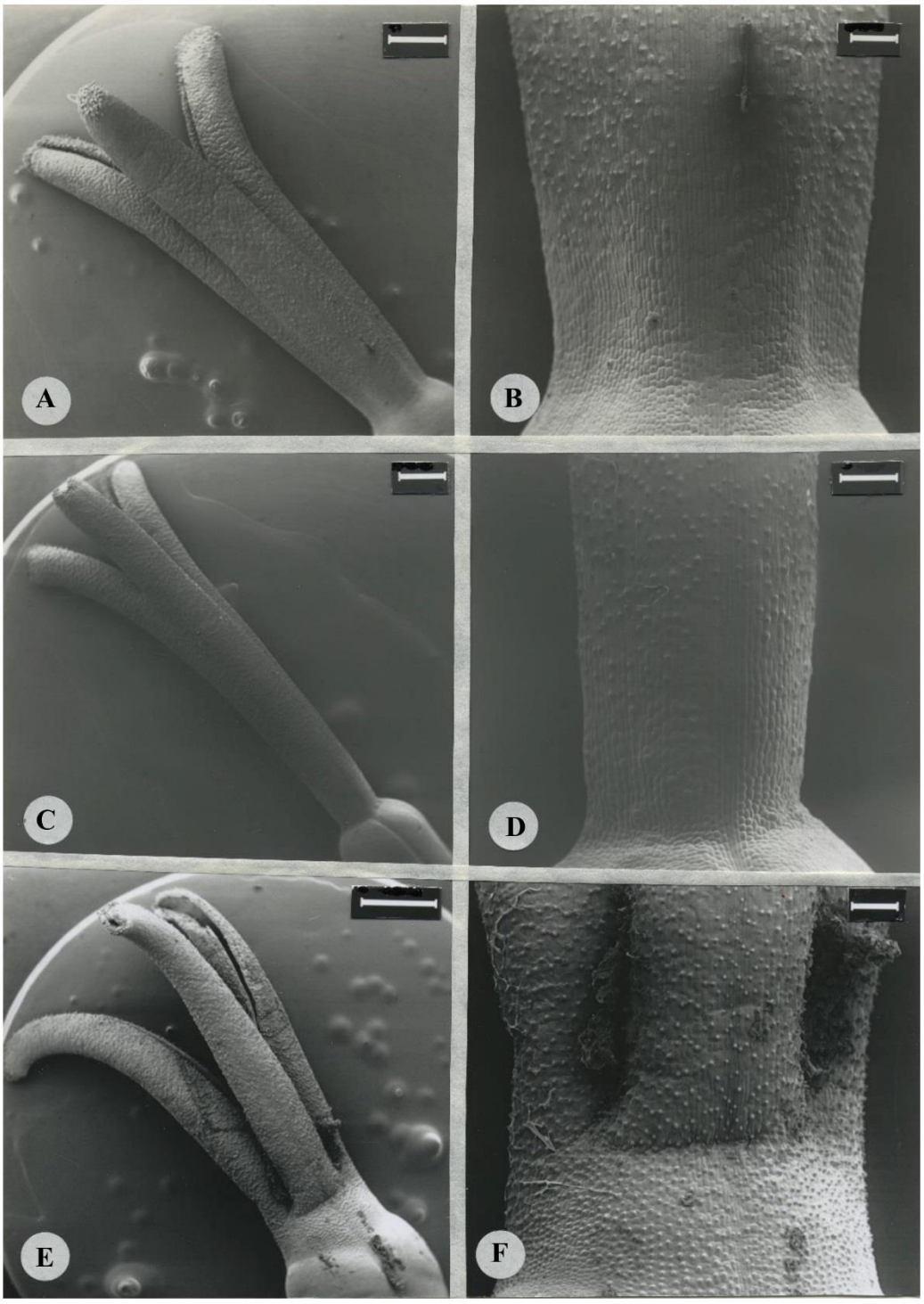

Fig. 3. SEM micrographs of branched styles in *Fritillaria michailovskyi*: A, style; B, style base. Branched styles in *Fritillaria reuteri*: C, style; D, style base. Branched styles in *Fritillaria straussii*: E, style; F, style base. Scales: A, D=1 mm; B, C, F=200 µm; E= 500 µm.

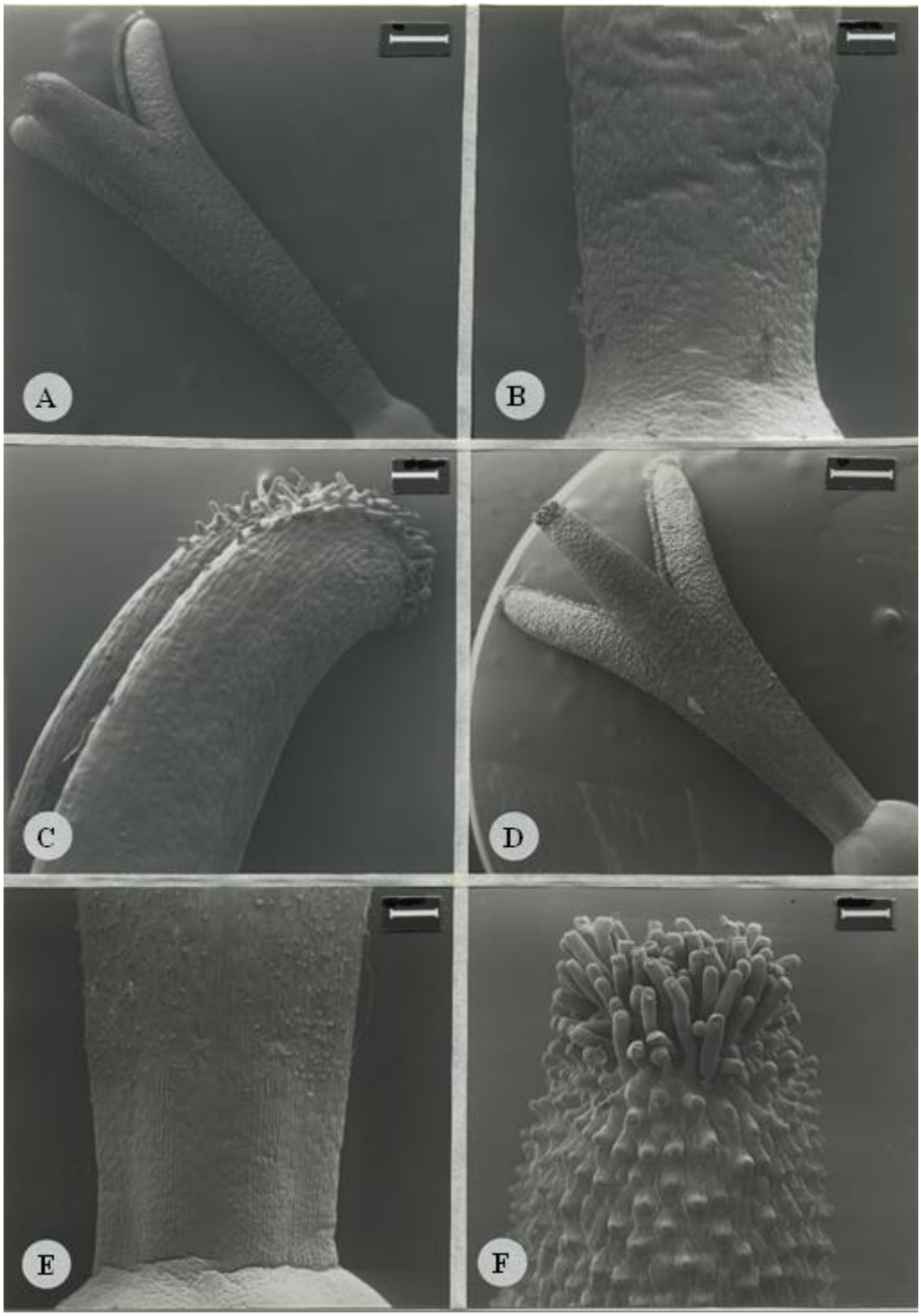

Fig. 4. SEM micrographs of glabrous branched type styles in *Fritillaria kotschyana* ssp. kotschyana: A, style; B, style base; C, a style branch. Minutely papillose branched type styles in *Fritillaria kotschyana* ssp. grandiflora: D, branched style; E, style base; F, stigma surface of a branch style. Scales: A & D=1 mm; B, C, E=200 µm; F=100 µm.

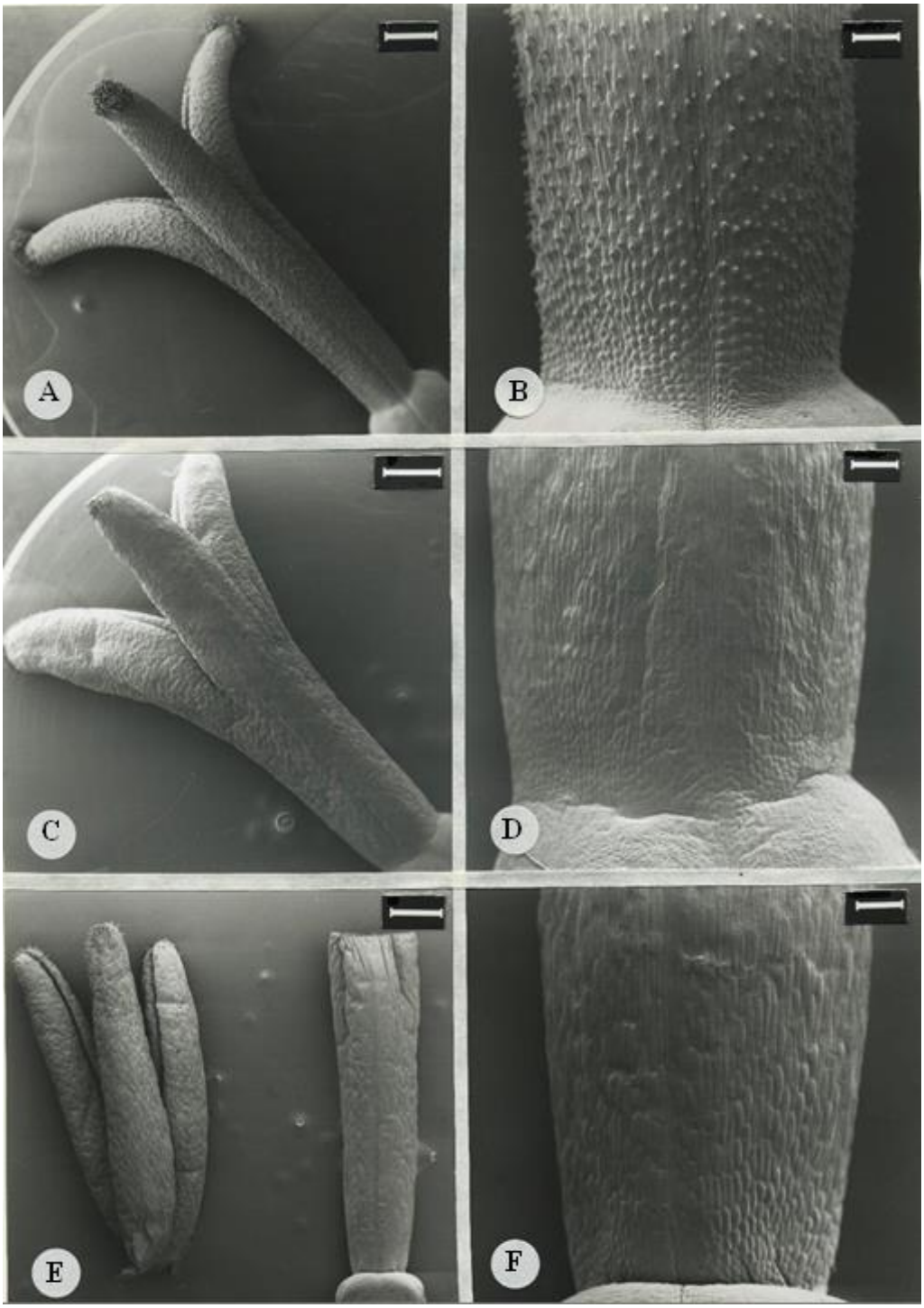

Fig. 5. SEM micrographs of papillose branched type styles in *Fritillaria hermonis* ssp. *amana*: A, style; B, style base. Glabrous branched type styles in *Fritillaria olivieri*: C, style; D, style base. Glabrous branched type styles in *Fritillaria whittallii*: E, style; F, style base. Scales: A, C, E=1 mm; B, D, F=200 µm.

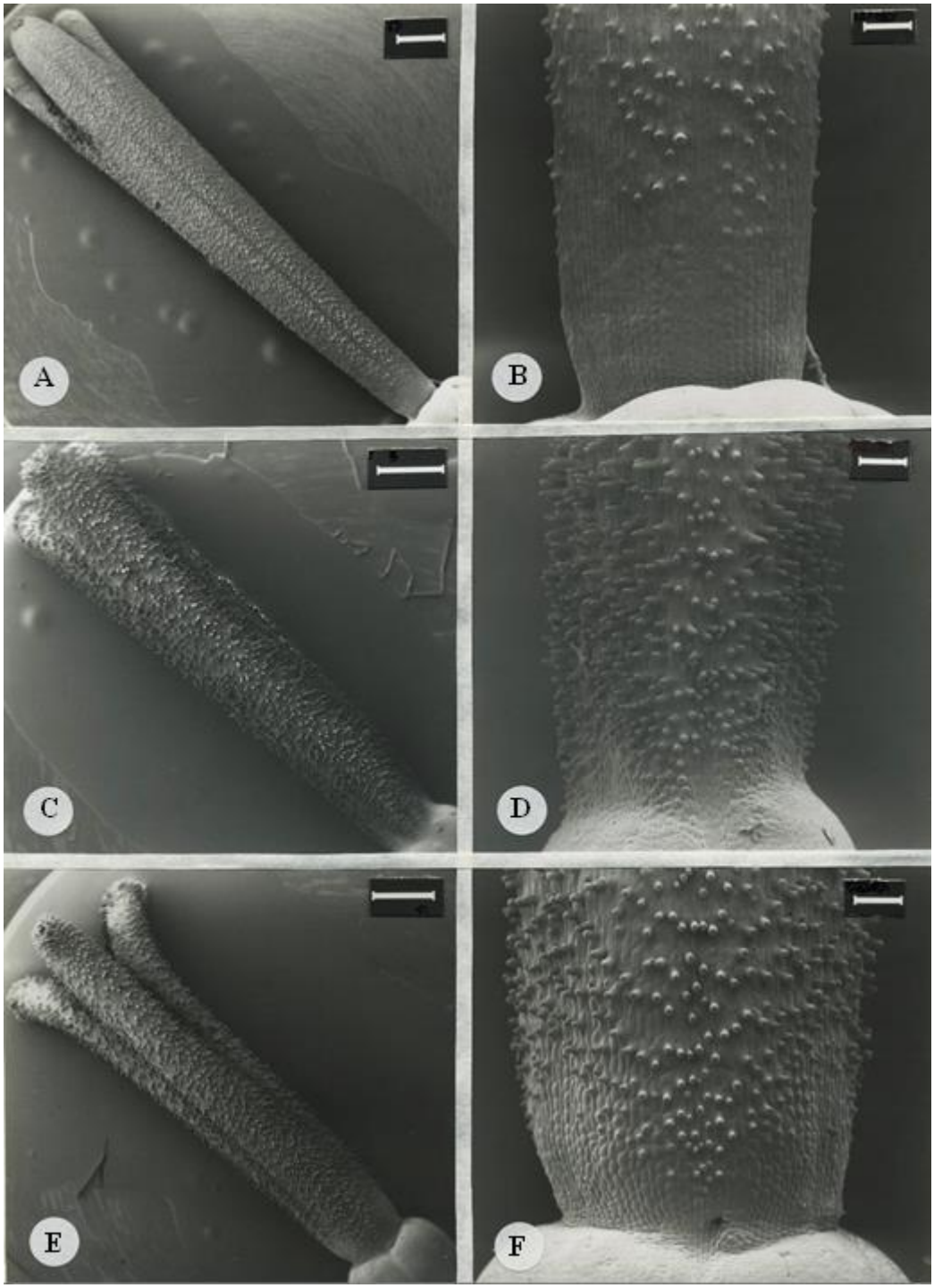

Fig. 6. SEM micrographs of papillose branched type styles in *Fritillaria caucasica*: A, style; B, style base. Densely papillose and branched type styles in *Fritillaria armena*: C, style; D, style base. Papilose branched type styles in *Fritillaria minima*: E, style; F, style base. Scales: A, C, E=1 mm; B, D, F=200 µm.

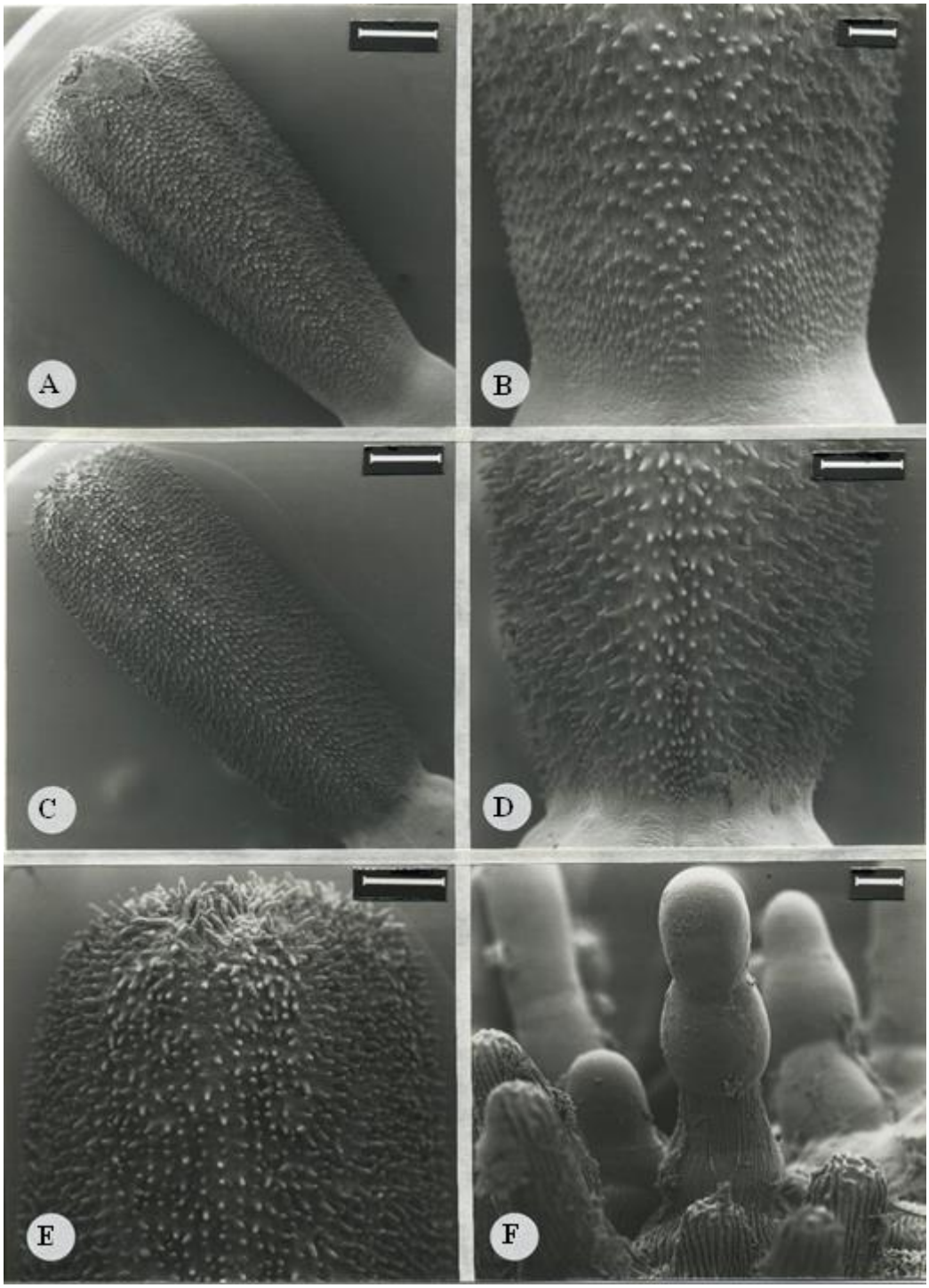

Fig. 7. SEM micrographs of densely papillose entire type of styles in *Fritillaria pinardii*: A, style; B, style base. Densely papillose entire type of styles in *Fritillaria uva-vulpis*: C, style; D, style base; E, style apex and stigma; F, cells on stigma surface. Scales: A & C=1 mm; B & F=200 µm; D & E=500 µm.

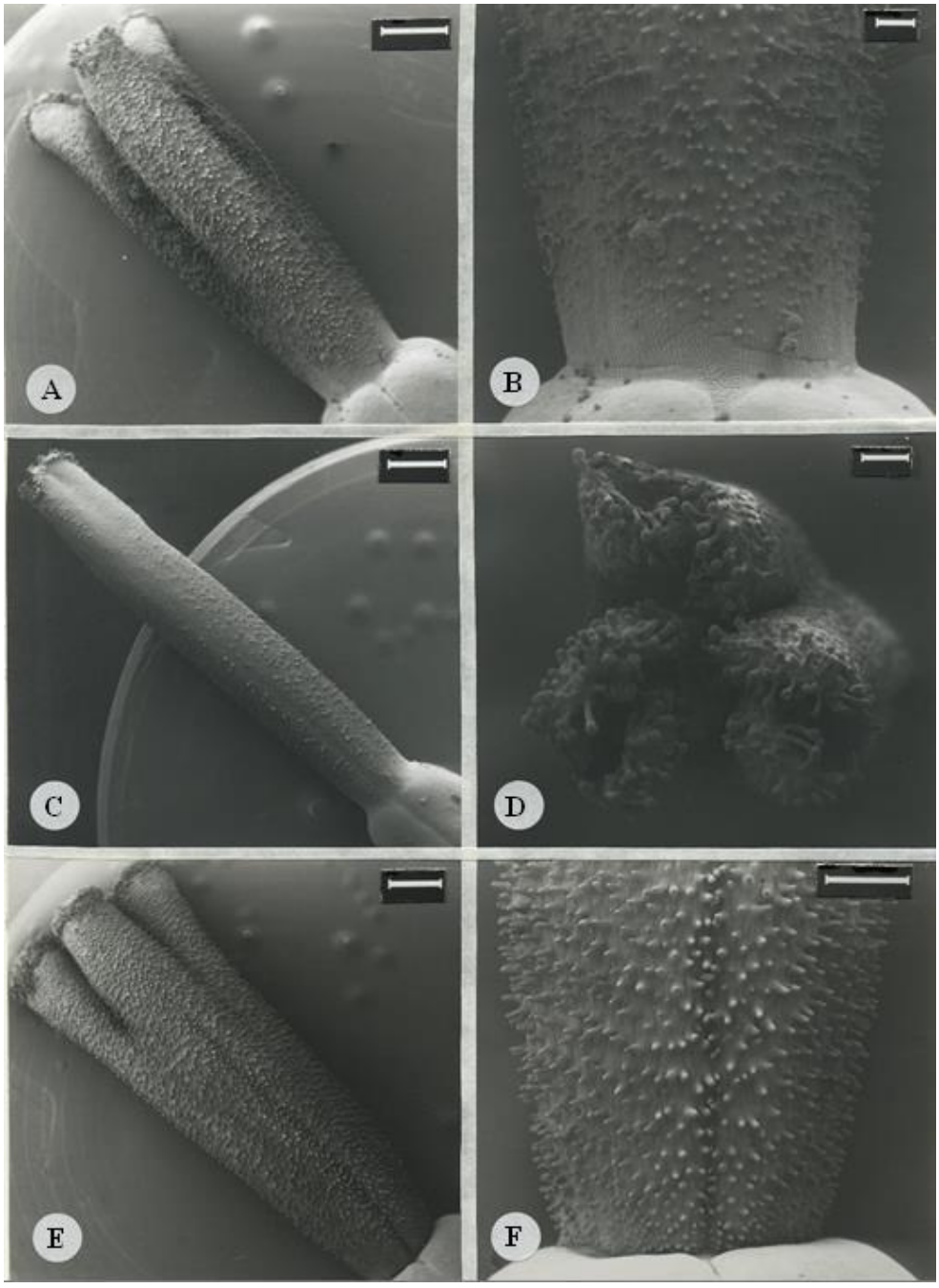

Fig. 8. SEM micrographs of papillose branched type styles in *Fritillaria chlorantha*: A, style; B, style base. Papillose branched type styles in *Fritillaria zagrica*: C, style; D, stigma surface. Densely papillose branched type styles in *Fritillaria assyriaca*: E, style; F, style base. Scales: A, C, E=1 mm; B & D=200 µm; F=500 µm.

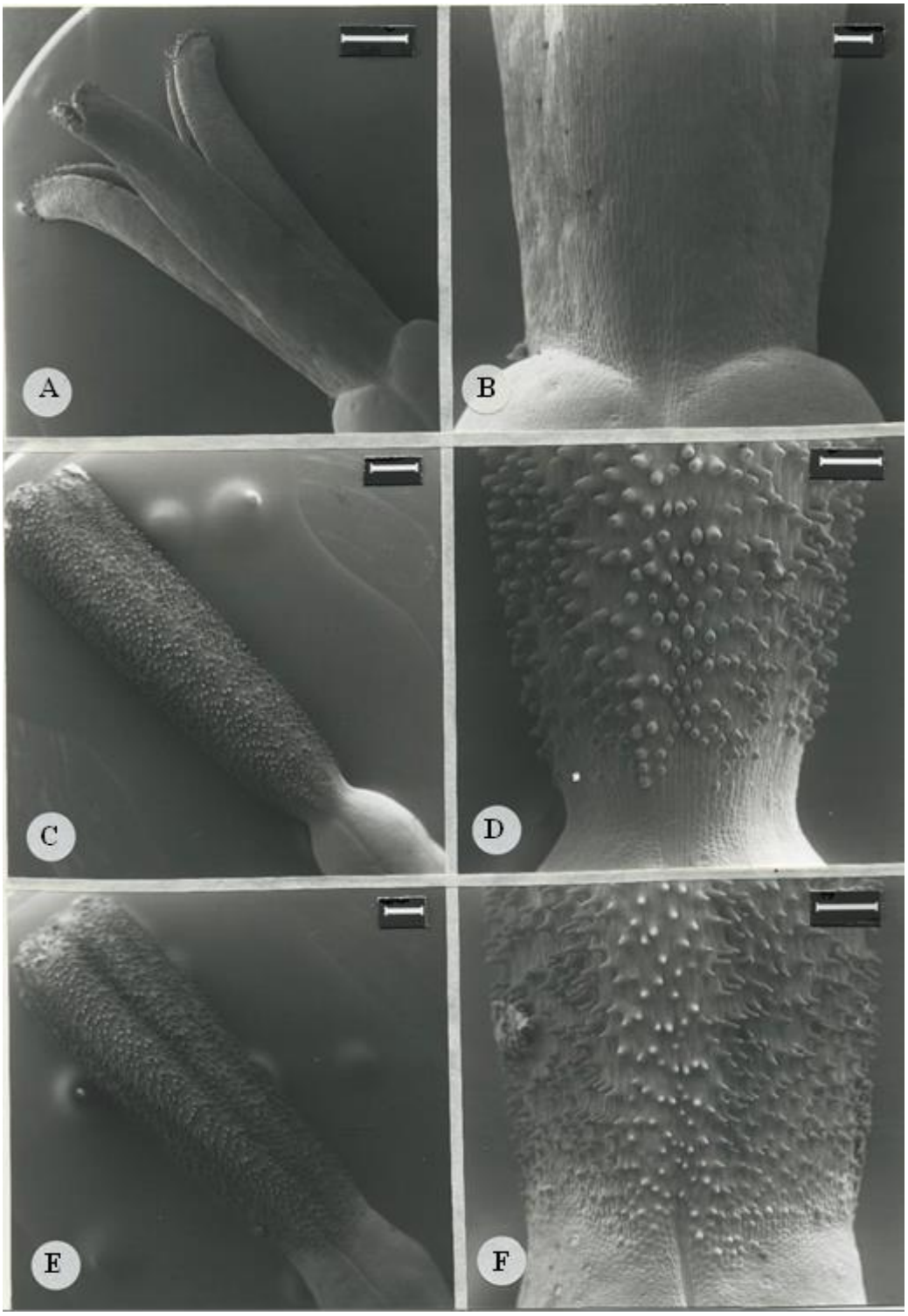

Fig. 9. SEM micrographs of glabrous branched type styles in *Fritillaria minuta*: A, style; B, style base. Densely papillose entire type styles in *Fritillaria atrolineata*: C, style; D, style base. Densely papillose entire type styles in *Fritillaria chlororhabdota*: E, style; F, style base. Scales: A, C, E=1 mm; B, D, F=200 µm.

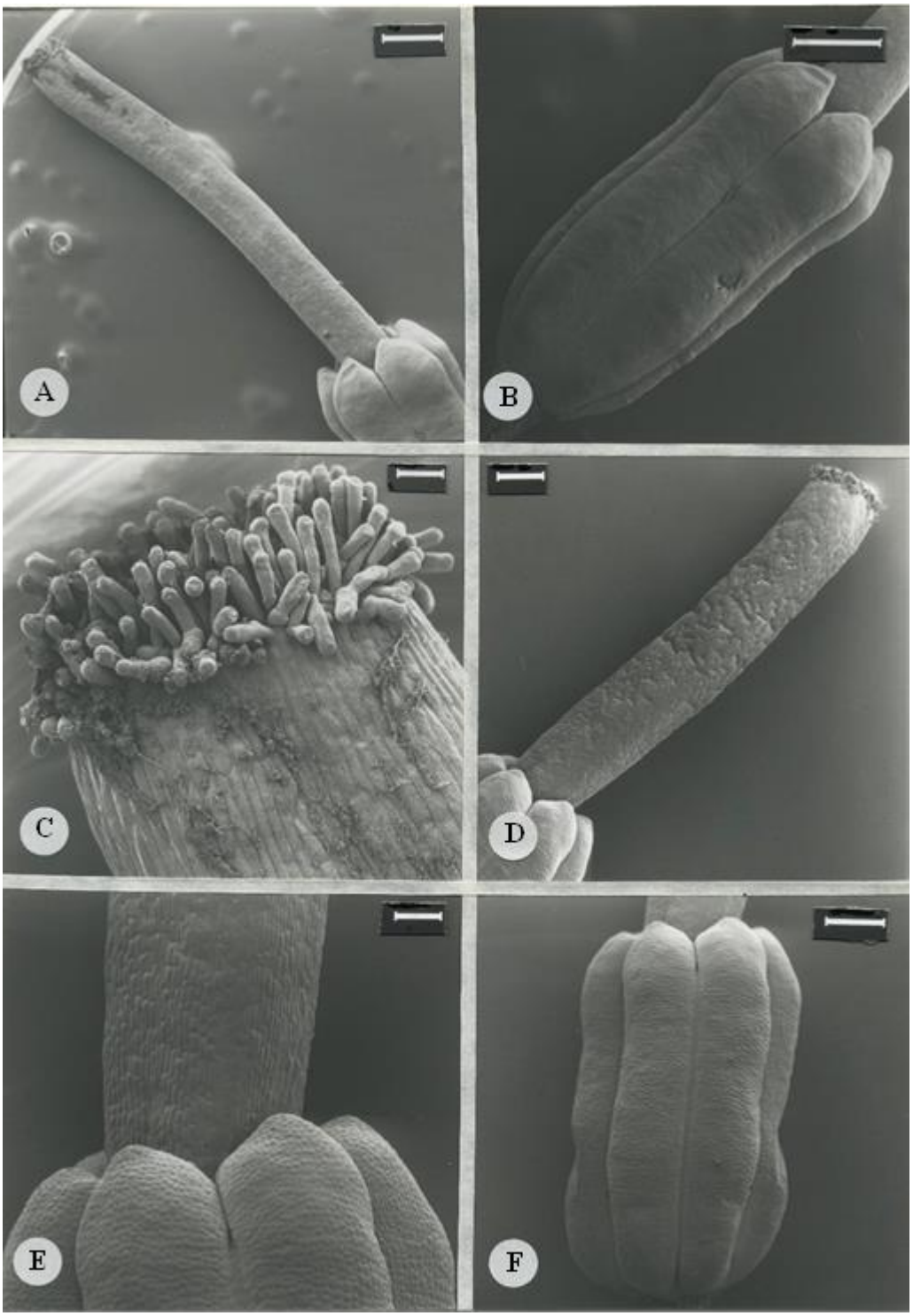

Fig. 10. SEM micrographs of glabrous entire type styles in *Fritillaria gibbosa:* A, style; B, cylindrical ovary and style base; C, stigma surface. Glabrous entire type styles in *Fritillaria aruana*: D, style; E, style base; F, cylindrical ovary. Scales: A & B=1 mm; C=100 µm; D & F=500 µm; E=200 µm.

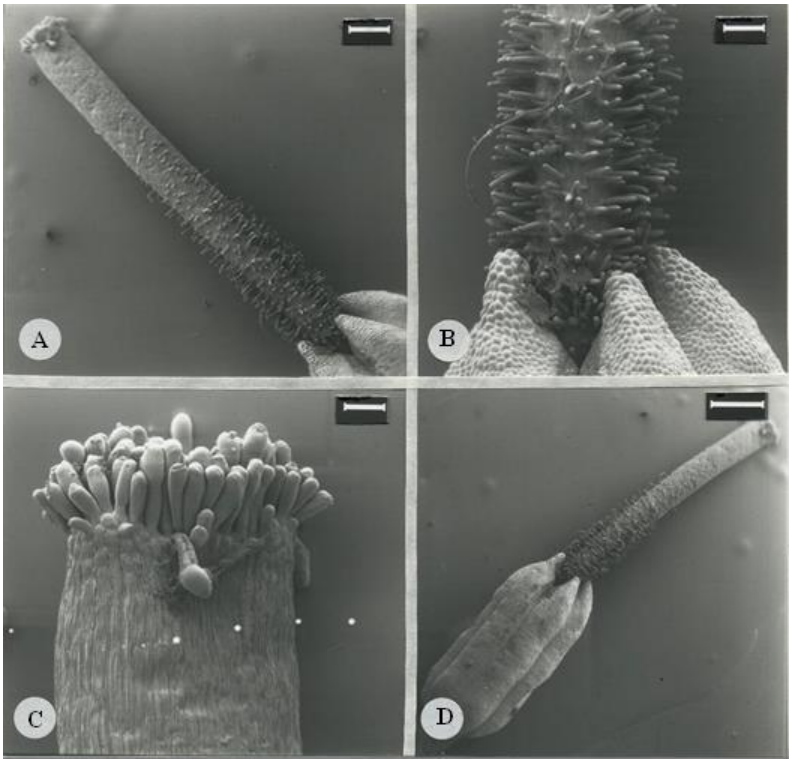

Fig. 11. SEM micrographs of densely papillose entire type styles in *Fritillaria stenantherum*: A, style; B, style base; C, stigma surface; D, ovary. Scales: A=500 µm; B=200 µ; C=100 µm; D=1 mm.

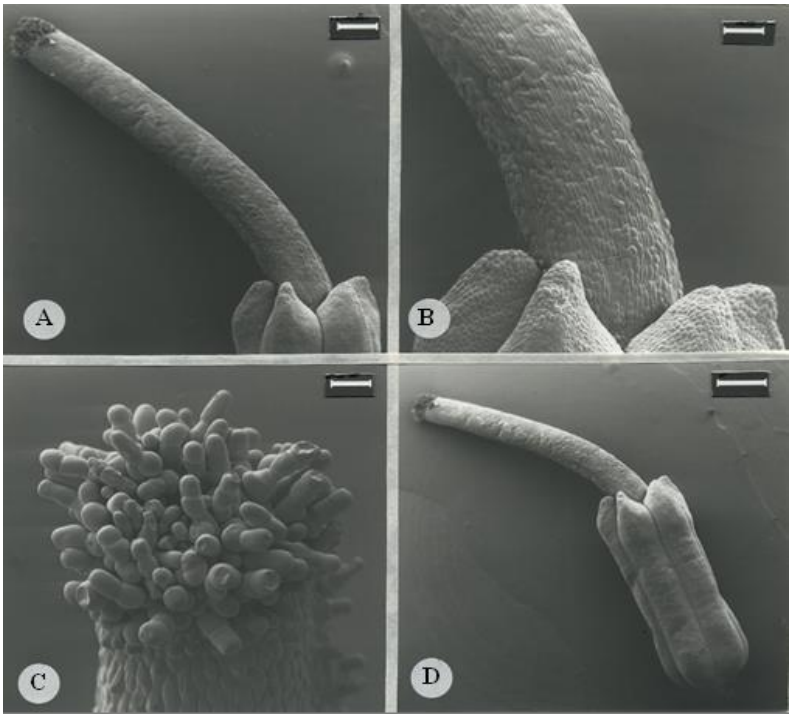

Fig. 12. SEM micrographs of glabrous entire type styles in *Fritillaria bucharica*: A, style; B, style base; C, stigma surface; D, ovary. Scales: A=500 µm; B=100 µ; C=200 µm; D=1 mm.

Table 4. Morphological qualitative characters of gynoecium in *Fritillaria* species.

No	**Taxon**	**Styles indumentum**	**Styles apex**	**Styles shape**	**Ovary shape**
1	*F. ariana*	1	1	1	3
2	*F. armena*	2	4	2	3
3	*F. assyriaca*	2	4	2	5
4	*F. atrolineata*	2	2	2	4
5	*F. bucharica*	1	1	1	1
6	*F. caucasica*	4	4	1	5
7	*F. chlorantha*	4	4	2	4
8	*F. chlororhabdota*	2	1	2	4
9	*F. crassifolia* ssp. *Crassifolia*	1	8	4	3
10	*F. crassifolia* ssp. *kurdica*	1	10	4	6
11	*F. gibbosa*	1	1	1	3
12	*F. hermonis*	4	6	3	4
13	*F. imperialis*	4	4	2	6
14	*F. karelinii*	3	1	1	3
15	*F. kotschyana* ssp. *grandiflora*	5	3	3	4
16	*F. michailovskyi*	4	7	4	7
17	*F. minima*	4	4	4	3
18	*F. minuta*	1	3	2	3
19	*F. olivieri*	1	9	3	4
20	*F. pinardii*	2	1	2	3
21	*F. poluninii*	5	8	4	6
22	*F. raddeana*	4	4	1	7
23	*F. reuteri*	4	3	4	4
24	*F. stenantherum*	2	1	1	2
25	*F. straussii*	4	6	2	6
26	*F. uva-vulpis*	2	1	3	3
27	*F. whittallii*	1	5	1	4
28	*F. zagrica*	4	1	1	3

Comparison between the phylogenetic tree based on the Mega-Tree of vascular plants (A), with the cladogram, provided from analysis of morphological features of the gynoecium (B) (Fig 13), shows that the monophyletic groups representing subgenera are consistent with groups in cladogram only for some subgenera like *Rhinoptalum* and *Petillum*, while dendrogram obtained from examining the characteristics of the androecium, including anthers and filament, was more consistent with the dendrogram obtained from the mega tree of the vascular plants in the studied subgenera (Mohsennezhad & al. 2024).

The morphological analysis of floral nectaries conducted by Bakhshi Khaniki (1997) and (Khaniki & al.1997) resulted in the reclassification of the *Rhinopetalum* group as a separate genus. Furthermore, Bakhshi Khaniki (2023) in the account for the flora of Iran, recognized the subgenus *Rhinopetalum* as an independent genus. The phylogenetic study does not support this status change (Rønsted & al. 2005). However, he explained the gynoecium characteristics as diagnostic traits for species delimitation.

For a more precise classification, it is recommended to use a combination of vegetative and reproductive characteristics along with molecular methods. However, the present study provided valuable data on the female reproductive organs and the importance of paying attention to the pollination mechanisms in *Fritillaria*. A pollination biology study may help to infer the relationship between style branching and the pollinators.

Table 5: Definition of the numbers used in Table 4 above (Terminology based on Rix1977)

Style indumentum	**Style apex**	**Style shape**	**Ovary shape**
1- Glabrous	1- Entire	1- Slender	1- Cylindrical-oblong
2- Densely papillose	2- Entire or very slightly 3-lobed	2- Stout	2- Oblong
3- Papillose only at apex(stigma)	3-3-fid for 2.5-4 mm long	3- Narrowly obconical stout and entire	3- Cylindrical
4- Papillose	4-3-fid for 1-2.5 mm long	4- Slender to narrowly stout	4- Stout
5- Minutely papillose toward apex	5-3-fid for 3-4.5 mm long		5- Obconial-cylindrical
	6- 3-fid almost from the base		6- Stout-cylindrical
	7-3-fid early to the middle with the branches 2-3.5 mm		7- Cylindrical and with rounded ribs
	8-3-fid for 1/2 of its length		
	9 - 3-fid for 1/3 to 1/4 of the length		
	10 - 3-fid for 1/4 to 1/5 of its length with branches 1-2 mm		

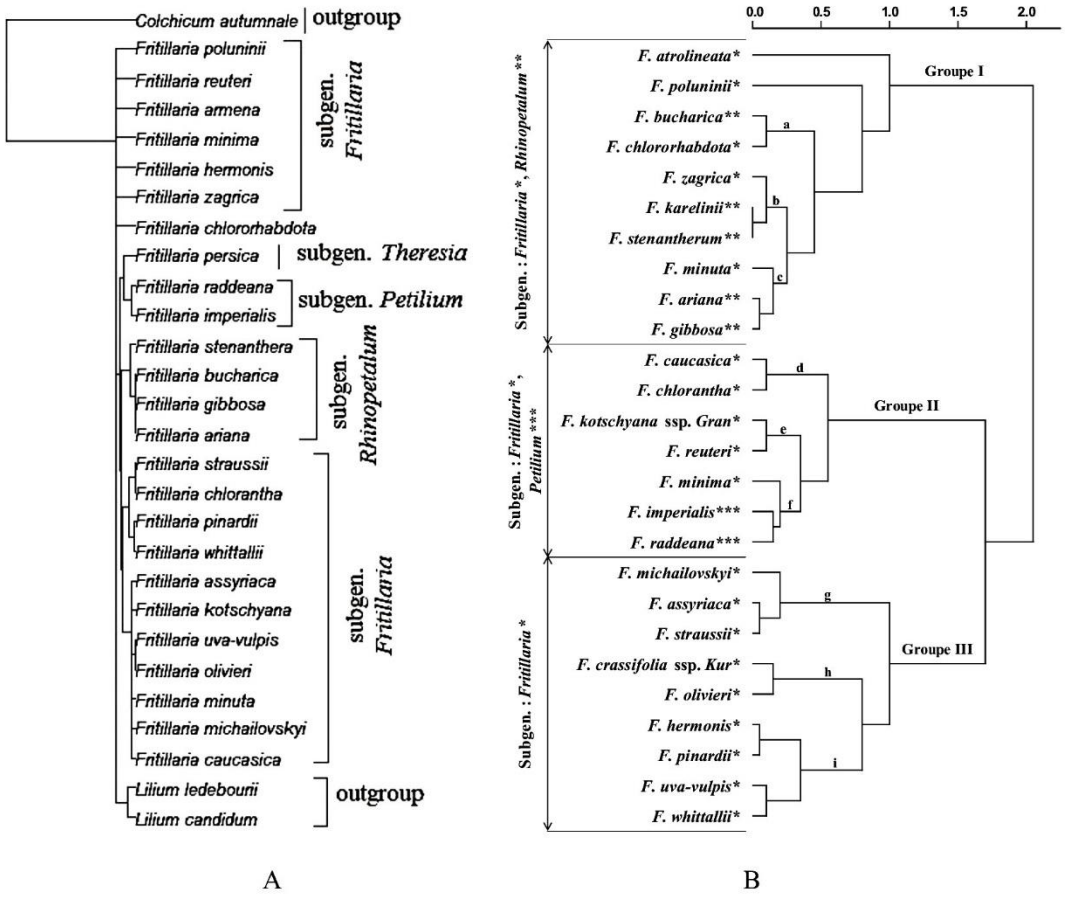

Fig 13. Comparison between phylogenetic tree based on Mega-Tree of vascular plants (A) and dendrogram based on morphological characters of gynoecium (R Core Team 2023) and Bray-Curtis dissimilarity (B) in *Fritillaria* species.

REFERENCES

Advay, M. & Sharifi-Tehrani M. 2016: Taxonomic Relationships of Ten Fritillaria Species of Subgenera *Fritillaria* and Theresia Based on Analysis of Flower Qualitative and Quantitative Morphological Characters. -Journal of Genetic Resources, 2: 11-22. https://doi.org/10.22080/jgr.2016.1476

Baker, J.G. 2009: Revision of the Genera and Species of Tulipeæ. -Botanical Journal of the Linnean Society, 14(76): 211-310. https://doi:10.1111/j.1095-8339.1874.tb00313.x

Bakhshi Khaniki, G. 1997: *Fritillaria atrolineata* (Liliaceae), a new species from Iran. -Edinburgh Journal of Botany, 54(2): 171-181.

Bakhshi Khaniki, G. 1998: Taxonomy and Karyology of the Genus *Fritillaria* S. Lat. (Liliaceae) in South West Asia with Special Reference to the Species in Iran. Gothenburg University Press. Sweden.

Bakhshi Khaniki, G. 2007: Taxonomic revision of the genus *Rhinopetalum* (Liliaceae) in Iran. -Plant Archives, 7: 47-52. https://doi :10.1111/j.1756-1051.1997.tb00355.x

Bakhshi Khaniki, G. 2023: Flora of Iran. Liliaceae. Nos. 171-175. Research Institue of Forests and Rengelands, Tehran.

Beetle, D.E. 1944: A Monograph Of The North American Species Of *Fritillaria*. -Madroño, 7(5): 133-159.

Boissier, E. 1884: Flora Orientalis, Vol: 5. Genevae. Basileae.

Darlington, C.D. & Wylie A.P. 1956: Chromosome atlas of flowering plants [by] C. D. Darlington [and] A. P. Wylie. London & Allen & Unwin.

Day, P.D., Berger, M. Hill, L. Fay, M.F., Leitch, A.R., Leitch, I.J. & Kelly, L.J. 2014: Evolutionary relationships in the medicinally important genus *Fritillaria* L. (Liliaceae). -Molecular Phylogenetics and Evolution 80: 11-19. https://doi.org/10.1016/j.ympev.2014.07.024

Hao, L., Fan, Y., da Silva, J.A.T. and Yu, X., 2023: Pollen morphology of 16 species of *Fritillaria* L. and its taxonomic implications. -Maejo International Journal of Science and Technology, 17(2): 123-137.

Khaniki, G.B. & Persson, K. 1997: Nectary morphology in South West Asian *Fritillaria* (Liliaceae). -Nordic Journal of Botany, 17(6): 579-611. https://doi.org/10.1111/j.1756-1051.1997.tb00355.x

Kiani, M., Mohammadi, S., Babaei, A., Sefidkon, F., Naghavi, M.R., Ranjbar, M., Razavi, S.A., Saeidi, K., Jafari, H., Asgari, D. & Potter, D. 2017: Iran supports a great share of biodiversity and floristic endemism for *Fritillaria* spp. (Liliaceae): A review. -Plant Diversity 39(5): 245-262. https://doi.org/10.1016/j.pld.2017.09.002

Komarov, V. L. 1935. *Fritillaria* L. In: Flora U.R.S.S. (Ed.) 4: 232-246. Akademiya Nauk SSSR.

Losina-Losinskaya, A.S. 1935: *Rhinopetalum* Fisch. In: V. L. Komarov (ed)., FI. U.R.S.S..

Mohsennezhad, F. & Bakhshi Khaniki, G. 2024; Systematic application of androecium morphology at the infra-generic classification of the genus *Fritillaria* (Liliaceae). -The Iranian Journal of Botany 30(2): 155-172. https://doi.org/10.22092/ijb.2024.366712.1484

Rechinger, K.H. 1990: In: Rechinger, K.H. (ed.), Flora Iranica, No. 165. Akademische Druck-u. Verlagsanstalt. Graz Austria.

Rix, E.M. 1975: Notes on *Fritillaria* (Liliaceae) in the eastern Mediterranean region, I & II. -Kew Bull. 29: 633-654. https://doi.org/10.2307/4108130

Rix, E.M. 1977: *Fritillarla* L.(Lillaceae) In IRAN. -The Iranian Journal of Botany 1(2): 75-96.

Rix, M., Frank, E., Webster, G. & Alpine, G. 2001: *Fritillaria*: a revised classification: together with an updated list of species. [England], *Fritillaria* Group of the Alpine Garden Society [England].

Rønsted, N., Law S., Thornton H., Fay, M.F. & Chase, M.W. 2005: Molecular phylogenetic evidence for the monophyly of *Fritillaria* and Lilium (Liliaceae; Liliales) and the infrageneric classification of *Fritillaria*. -Molecular phylogenetics and evolution 35(3): 509-527. https://doi.org/10.1016/j.ympev.2004.12.023

Tekşen, M. & Aytaç, Z. 2011: The revision of the genus *Fritillaria* L.(Liliaceae) in the Mediterranean region (Turkey). -Turkish Journal of Botany 35(5): 447-478.

Zhang, Z., Xing, C., Su, H., Wang, J., Qi, Y. & Li, M. 2024: In vitro plant regeneration and bioactive metabolite production of endangered medicinal plant *Fritillaria* cirrhosa. -*Current Plant Biology*, p.100363.