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Review

A Review on the Composition and Biosynthesis of Alkaloids and on the Taxonomy, Domestication, and Cultivation of Medicinal *Fritillaria* Species

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Abstract: *Fritillaria* is a perennial herb with medicinal properties. There are 158 *Fritillaria* species worldwide, 33 of which have reported therapeutic efficacy. Alkaloids are the principal constituents in *Fritillaria*. *Fritillaria* species growing at 2700–4000 m are the sources of extract namely Chuan Beimu (the Pharmacopoeia of the People's Republic of China, 2020 Edition), with low biomass, mainly containing more 5α-cevanine isosteroidal alkaloids with *cis*-configuration. In contrast, species growing below 1500 m are usually taller than 50 cm, and they mainly contain more *trans*-configuration isosteroidal alkaloids. There are two schemes of the biosynthetic pathways of steroidal alkaloids with different frameworks and catalytic reactions and combined high-throughput omics data. Based on the distributed elevations, *Fritillaria* species were divided into three major categories, which met classification features based on phylogenetic analysis or morphological features. Artificial or in vitro cultivations are effective strategies for balancing economical requirements and ecological protection. *Fritillaria* species growing at lower altitudes can be cultivated by bulb reproduction, but species growing at higher altitudes still rely mainly on gathering a large number of wild resources. Integration of asexual tissue culture and bulb reproduction with sexual artificial or imitated wild cultivation may create a very promising and effective way to maintain sustainable industrial development of *Fritillaria*.

Keywords: *Fritillaria*; alkaloid composition; phytochemical biosynthesis; taxonomy; domestication; cultivation

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1. Introduction

Traditional Chinese medicine (TCM) has used the bulbus *Fritillariae* for thousands of years [1]. The nature of *Fritillariae* is slightly chilly, making it useful for removing heat, moistening the lungs, and lowering fever, according to TCM theory [2–4]. *Fritillariae* is also used to treat under-the-skin tumors including scrofulous swellings and breast lumps [2]. According to their morphology, molecular markers, and major pharmacological activities, the Pharmacopoeia of the People's Republic of China (2020 Edition) classifies five species of dry bulbus *Fritillariae* as Beimu [5]. A dry cough owing to lung heat and a taxation cough due to yin deficiency are cured using Chuan Beimu (*F. cirrhosa* D. Don, *F. unibracteata* Hsiao et K. C. Hsia, *F. przewalskii* Maxim., *F. delavayi* Franch., *F. taipaiensis* P. Y. Li, and *F. unibracteata* Hsiao et K. C. Hsiavar. wabuensis (S. Y. Tanget S. C. Yue) Z. D. Liu., S. Wang et S. C. Chen. Jiang et al.), Ping Beimu (*F. ussuriensis* Maxim), and Yi Beimu (*F. walujewii* Regel and *F. pallidiflora* Schrenk) [2,5]. The geo-authentic bulbus *F. cirrhosae* D. Don have been used as an ingredient in more than 200 traditional Chinese prescriptions such as the drug Nin Jiom Pei Pa Koa [6]. *F. ussuriensis* naturally grows in the Northeast region of China, and it is the main ingredient of the Chinese patented "Fufang Beimu Tablets" for relieving cough and reducing sputum [7]. *F. walujewii* and *F. pallidiflora* as the botanical origins of Yi Beimu are the main ingredients in the prescription medicine "Qiuzao Ganmao Granules" [8]. *F. thunbergii* Miq. (Zhe Beimu) grows in the Zhejiang Province of China. Its bulbus is utilized as a prime ingredient in various herbal formulae, including the compound medicine known as "Danggui Beimu Kushen Wan" because it is particularly effective at preventing a cough, eliminating phlegm, and hemostasis [9]. Oral liquids of *F. hupehensis* Hsiao et K. C. Hsia decoction are used for relieving phlegm heat coughs [10]. Chuan Beimu, Zhe Beimu and Hubei Beimu are also effective at treating carbuncle [10].

Isosteroidal alkaloids and steroidal alkaloids have been identified as the active ingredients in *Fritillaria* (Figure 1 and Figures S1–S7) [10,11]. According to Hao et al. [2], several chemical compositions of 10 different *Fritillaria* species were proposed, and these species were divided into two groups depending on whether they contained *trans*- or *cis*-cevanine alkaloids. Simultaneously, the evolutionary connection between the several *Fritillaria* species was inferred using both a nuclear internal transcribed spacer (ITS) and chloroplast *matK* sequences. Traditional uses, 72 phytochemical profiles, and the pharmacological properties of *F. thunbergii* were summarized in Nile et al. [4]. Meanwhile, 182 chemical compounds from the genus *Fritillaria*, including alkaloids, terpenoids, and other compounds had their structures, traditional applications, and pharmacology elucidated [12]. Soon afterwards, 293 chemical profiles and analytical methodologies for phytochemical composition of *Fritillaria* species were covered in depth by Wang et al. [13].

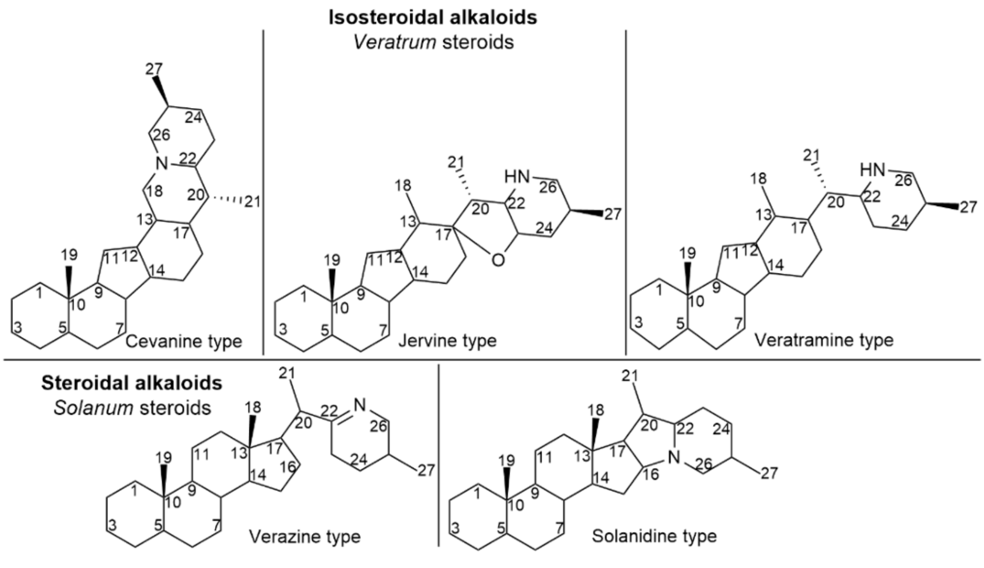


Figure 1. The types of steroidal alkaloids in *Fritillaria* species.

Herein, we present an interdisciplinary and a multi-perspective review on the composition and biosynthetic pathways of alkaloids, with a taxonomy based on classical methods and molecular decoding, domestication, and cultivation for medicinal *Fritillaria* species. This review will systematically expound on the composition, synthesis, and regulation of alkaloids in *Fritillaria* species. Additionally, alkaloids from various base sources will be contrasted. Medicinal *Fritillaria* species will be categorized based on distributed elevations, phylogenetic analysis, and morphological features. Various classification results will be compared and analyzed. Finally, this review will introduce several effective techniques and methods for the domestic cultivation and protection of *Fritillaria* species. Simultaneously, we also provide insights into *Fritillaria* species for further research.

2. Composition and Biosynthesis of Alkaloids in *Fritillaria* Species

Fritillaria is rich in various secondary metabolites. The identification and isolation of various chemical compounds in *Fritillaria* have been conducted using several methods and techniques, including ultra-performance liquid chromatography (UPLC), mass spectrometry (MS), nuclear magnetic resonance (NMR), and supercritical fluid extraction (SFE) [14–18]. Particularly, the advanced technique of ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) based untargeted metabolomics coupled with chemometric analysis has been successfully applied to the accurate identification of major components [14,19], such as alkaloids, terpenoids, nucleosides, organic acids, saponins, carbohydrates, amines, and sterols in various Chinese herbs [1,2]. Undoubtedly, characterization of major bioactive ingredients and verification of their pharmacological activities is crucial for quality evaluation and control of various bulbus *Fritillariae* with different origins [3].

2.1. The Composition of Alkaloids in Fritillaria Species

Alkaloids possess cyclic structures containing at least one six-membered carbon ring embedded by one basic nitrogen atom, and they are regarded as valuable markers in *Liliaceae* [13]. So far, more than 100 alkaloids have been isolated from different parts of *Fritillaria* species (Table 1 and Figures S1–S7). Based on their structural frameworks, the alkaloids extracted from *Fritillaria* species can be classified as isosteroidal and steroidal types [2,20]. Subsequently, the isosteroidal type of alkaloids are further sub-divided into three types: Cevanine, Jervine, and Veratramine, according to the patterns of linkage between the E and F rings (Figure 1) [2,13]. On the other hand, the steroidal type of alkaloids can be sub-divided into two types: Verazine and Solanidine, depending on the nitrogen atom to be incorporated into an indolizidine ring or a piperidine ring (Figure 1) [2,21].

Table 1. The main morphological features and the types of alkaloids of medicinal *Fritillaria* species.

Species	The Number of Main Bulbs	Bulb Diameter (cm)	Plant Height (cm)	Florescence	Habitat Types and Specimen Collection Places
Group 1 (approximately 2700–4000 m)					
F. cirrhosa	2	1–1.5	15–65	May–July	Under forests, in alpine thickets, or on meadows and flood lands. The growth altitude ranged from 2500 to 4600 m. The regions: Sichuan, Xizang, and Yunnan in China; Nepal; India
	The types of alkaloids	Cevanine type with *cis*-configuration (8): imperialine, chuanbeinone, imperialine-β-N-oxide, delavine, 3β-acetylimperialine, delavinone, isodelavine, yibeinoside A [22–25]. Cevanine type with *trans*-configuration (6): peimine, peiminine, puqiedine, ebeiedinone, ebeiedine, isoforticine [22,23,25]. Jervine type (2): peimisine-3-*O*-β-D-glucopyranoside, peimisine [22,24]. Veratramine type (1): puqienine B [22]. Verazine type (4): puqietinone, cirrhosinine A, cirrhosinine B, delavidine [22,23]. Solanidine type (4): solanidine, solanidine-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside, demissidine, demissidine-3-*O*-β-D-glucopyranosyl (1→4) glucopyranoside [22,24,26].			
F. unibracteata	2	0.6–0.8	15–50	June	In moist places of thickets, or on meadows. The altitude ranged from 3200 to 4700 m. The regions: Sichuan and Qinghai in China.
	The types of alkaloids	Cevanine type with *cis*-configuration (6): chuanbeinone, imperialine, delavinone, delavine, yibeinoside A, imperialine-3β-D-glucoside [27–29] Cevanine type with *trans*-configuration (5): ebeiedinone, peiminine, isopeimine, peimine, puqiedinone-3-*O*-β-D-glucopyranoside [28,29]. Jervine group (3): songbeisine, peimisine, peimisine-3-*O*-β-D-glucopyranoside [28–30].			
F. taipaiensis	2	1–1.5	20–100	May–July	Under forests, in hill thickets, or on grassy slopes. The growth altitude ranged from 1500 to 3200 m. The regions: Shaanxi, Gansu, Sichuan, and Hubei in China.
	The types of alkaloids	Cevanine type with *cis*-configuration (4): taipaienine, chuanbeinone, imperialine, taipainine D [31–34]. Cevanine type with *trans*-configuration (2): peimine, peiminine [31,32]. Jervine type (2): peimisine, taipainine A [32,34,35].			
F. delavayi	2–3	1–2	35	June–July	In sandy and gravelly places or on flood lands. The growth altitude ranged above 4000 m. The regions: Yunnan, Sichuan, Qinghai, and Xizang in China.
	The types of alkaloids	Cevanine type with *cis*-configuration (5): chuanbeinone, yibeinoside A, imperialine, delavine, delavinone [36,37]. Cevanine type with *trans*-configuration (2): peimine, peiminine [36,37]. Jervine type (1): peimisine [36]. Verazine type (1): delavidine [37].			

Table 1. *Cont.*

Species	The Number of Main Bulbs	Bulb Diameter (cm)	Plant Height (cm)	Florescence	Habitat Types and Specimen Collection Places
F. crassicaulis	2	2–2.5	30–60	May	Under forests, or in alpine thickets. The growth altitude ranged from 2500 to 3500 m. The region: Yunnan in China.
F. przewalskii	2	0.6–1.3	20–40	June–July	In thickets or on meadows. The growth altitude ranged from 2800 to 4400 m. The regions: Gansu, Qinghai, and Sichuan in China.
Group 2 (approximately 1500–2700 m)					
F. pallidiflora	2	1.5–3.5	30–60	May	In thickets, or on meadows. The growth altitude ranged from 1300 to 1780 m. The region: Xinjiang Uygur Autonomous region in China.
	The types of alkaloids	Cevanine type with *cis*-configuration (12): imperialine, imperialine-3β-D-glucoside, imperialine-β-N-oxide, yibeinoside A, delavine, yubeinine, sinpeinine A, delavinone, chuanbeinone, 5α, 14α, 17β-cevanin-6-oxo-3β, 20β, 24β-triol, 17β-cevanin-6-oxo-5α,20β-diol, yibeinine [38–46]. Cevanine type with *trans*-configuration (7): yibeinone C, yibeinone D, dongbeinine, zhebeinone-3β-D-glucoside, peimine, yibeinone E, yibeirine [41,42,45]. Other cevanine type (1): ebeinone [45]. Jervine type (6): peimisine, yibeissine, cyclopamine, cycloposine, (20R,22R,23R,25R)-3β,23-dihydroxy-N-methyl-veratram-13(17)-en-6-one, yibeinone A [39,42,47,48]. Veratramine type (1): yibeinone B [42]. Verazine type (2): pingbeinine, yibeinoside C [41]. Solanidine type (4): avenacoside C, (25R)-26-[β-D-glucopyranosyl]oxy]-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)oxy]-cholesta-5,17-diene-16,22-dione, 26-*O*-β-D-glucopyranosyl-3,26-dihydroxy-(25R)-5β-furost-12-on-20(22)-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, aspidistrin [48].			
F. walujewii	2	1–1.5	20–40	May–June	In thickets, on meadows, or in the cracks of rocks. The growth altitude ranged from 1300 to 2000 m. The regions: Xinjiang Uygur Autonomous region in China; Russia.
	The types of alkaloids	Cevanine type with *cis*-configuration (8): tortifoline, imperialine-3β-D-glucoside, imperialine, yibeinoside A, walujewine B, walujewine C, walujewine D, walujewine E [49,50]. Cevanine type with *trans*-configuration (2): petilidine, ebeiedine [50,51]. Jervine group (3): walujewine A, songbeisine, peimisine [50].			

Table 1. *Cont.*

Species	The Number of Main Bulbs	Bulb Diameter (cm)	Plant Height (cm)	Florescence	Habitat Types and Specimen Collection Places
F. karelinii	2	1	12–35	April	In Artemisia desert or on ferulic beach. The growth altitude ranged from 590 to 3150 m. The region: Xinjiang Uygur Autonomous region in China.
	The types of alkaloids	Cevanine type with *cis*-configuration (1): persicanidine B [52]. Other cevanine type (3): 27-epiebeienine, ebeienine, heilonine [52]. Jervine type (2): karelinine, 5-epikarelinine [52].			
F. maximowiczii	4–5	1–2	27–54	June	On the hillsides. The growth altitude ranged from 1400 to 1480 m The regions: Hebei, Liaoning, and Jilin in China.
	The types of alkaloids	Jervine type (2): kuroyurinidine, 23-isokuroyurinidine [53].			
F. davidii	3–4	1–2	10–35	April	On meadows, or in the cracks of rocks. The growth altitude ranged from 1800 to 2300 m. The region: Sichuan in China.
F. verticillata	2	2	40–50	April–July	On the hillsides. The growth altitude ranged at 1600 m. The regions: Xinjiang Uygur Autonomous region in China; Japan.
	The types of alkaloids	Cevanine type with *trans*-configuration (2): fritillarizine, isobaimonidine [54,55].			
F. tortifolia	2–3	1–3	20–40	April–May	In alpine thickets or on grassy slopes. The growth altitude range was: 1500–2000 m. The region: Xinjiang Uygur Autonomous region in China.
	The types of alkaloids	Cevanine type with *cis*-configuration (9): tortifoline, frititorine A, frititorine B, imperialinol, imperialine, yubeinine, imperialine-3β-D-glucoside, delavinone, hupehenizioiside [56,57]; Cevanine type with *trans*-configuration (2): ebeinine, ebeiedinone [57]; Jervine type (3): frititorine C, peimisine, peimisine-3-*O*-β-D-glucopyranoside [57].			
F. meleagroides	2	0.5–1.5	40	April	In thickets, on meadows, or on flood lands. The growth altitude range was: 1500 m. The region: Xinjiang Uygur Autonomous region in China; Kazakhstan.

Table 1. *Cont.*

Species	The Number of Main Bulbs	Bulb Diameter (cm)	Plant Height (cm)	Florescence	Habitat Types and Specimen Collection Places
Group 3 below 1500 m					
F. thunbergii	2–3	1.5–3	50–80	March–April	Low altitude hill under partial shade. The regions: Zhejiang, Anhui, Jiangsu, Jiangxi, and Hunan in China; Japan.
	The types of alkaloids	Cevanine type with *trans*-configuration (15): peimine, peimidine, peiminine, zhebeinine, eduardine, zhebeirine, ebeiedine, puqiedine, fritillarizine, isobaimonidine, isopeimine, zhebeininoside, peiminoside, verticine-N-oxide, ebeiedinone [3,4,58–62]. Jervine type (2): cyclopamine, peimisine [4,63]. Veratramine type (1): zhebeisine [64]. Verazine type (2): N-demethylpuqietinone, fetisinine [64]. Solanidine type (2): solanidine, solanidine [65].			
F. monantha	2–3	1.2–3	60–100	April–June	Under forests, in water side, or on wetlands. The growth altitude ranged from 700 to 1200 m. The regions: Hubei, Henan, Anhui, Zhejiang, and Jiangxi in China.
	The types of alkaloids	Cevanine type with *trans*-configuration (6): peimine, peiminine, hupeheninoside, isopeiminine, 3-*O*-acetoxyverticinone, 3-*O*-acetylverticine [66–70]. Cevanine type with *cis*-configuration (1): delavine [66,69]. Jervine type (4): pengbeimine B, pengbeimine D, peimisine, ebeiensine [71,72]. Veratramine type (2): (3β, 5α, 13α, 23β)-7, 8, 12, 14-tetradehydro-5, 6, 12, 13-tetrahydro-3, 23-dihydroxyveratraman-6-one, (3β, 5α, 13α, 23β)-7, 8, 12, 14-tetradehydro-5, 6, 12, 13-tetrahydro-3, 13, 23-trihydroxyveratraman-6-one [69].			
F. anhuiensis	2–3	2	50	March–April	Under forests. The growth altitude ranged from 300 to 1500 m. The region: Anhui in China.
	The types of alkaloids	Cevanine type with *trans*-configuration (6): wanpeinine A, peimine, peiminine, isopeimine, ebeiedinone, verticinedinone [73,74]. Jervine type (4): peimisine, pengbeimine A, pengbeimine B, pengbeimine D [74]. Verazine type (1): sitosterol [74]. Solanidine type (2): solanidine, (22S,25S)-solanid-5,20(21)-dien-3beta-ol [75].			
F. ussuriensis	2	1–1.5	100	May–June	In thickets, on meadows, or in river valleys. The growth altitude ranged at low elevations. The regions: Liaoning, Jilin, and Heilongjiang in China; Russia, Korea.
	The types of alkaloids	Cevanine type with *trans*-configuration (7): pingpeimine A, pingpeimine C, peimine, peiminine, ebeiedinone, eduardine, isopeimine [76–79]. Cevanine type with *cis*-configuration (3): pingpeimine B, delavine, imperialine [78,79]. Other Cevanine type (5): ussuriedine, ussurienine, ussurienone, ussuriedinone, heilonine [77,79,80]. Jervine type (1): peimisine [76]. Veratramine type (1): pingbeimunone A [77]. Verazine type (2): pingbeinine, pingbeidinoside [81].			

Note: The structures of these mentioned alkaloids are listed in Figures S1–S7.

As previously reported, 5α-cevanine isosteroidal alkaloids are the pharmaceutical active ingredients of the *Fritillaria* genus [10,11]. By analyzing the composition of 5α-cevanine isosteroidal alkaloids in *Fritillaria* species, some regularities were found (Table 1). *F. thunbergii* and *F. anhuiensis* S. C. Chen and S. F. Yin only contained the 5α-cevanine isosteroidal alkaloids with *trans*-configuration (i.e., the two H atoms of C-13 and C-17 at the state of *trans*-configuration), including peimine (verticine), peiminine (verticinone), isoverticine, and ebeiedine (Figure S1), as well as *F. ussuriensis* Maxim and *F. monantha* Migo mainly contained 5α-cevanine isosteroidal alkaloids with *trans*-configuration. Alternatively, *F. cirrhosa*, *F. unibracteata*, *F. walujewii* and *F. delavayi* mainly contained more isosteroidal alkaloids with a *cis*-configuration (i.e., the two H on C-13 and C-17 at the state of *cis*-configuration), including imperialine, chuanbeinone, and taipaienine among others (Figure S2). It was reported that the rank order of potency was imperialine > peimine > peiminine > ebeienine [82]. Moreover, *F. maximowiczii* mainly contained Jervine group alkaloids instead of either *trans*- or *cis*-configuration alkaloids (Figure S4). Importantly, 5α-cevanine isosteroidal alkaloids are key quality control indicators for several bulbus *Fritillariae*. Peimine and peiminine are the main alkaloids in the bulbus *F. thunbergii* [12,83], with the latter being a key quality control indicator for *F. ussurensis* and *F. monantha* [3,58]. Further, imperialine (sipeimine) is used for quality assessment of Chuan Beimu. The combination of imperialine and imperialine-3β-D-Glu is also used as the analytical index for *F. pallidiflora* and *F. walujewii* [5].

2.2. Comparison of Alkaloids in Fritillaria from Different Base Sources

There are six species of the *Fritillaria* genus used as the botanical origins of Chuan Beimu in the Chinese Pharmacopoeia (2020 Edition), including *F. cirrhosa*, *F. unibracteata*, *F. przewalskii*, *F. delavayi*, *F. taipaiensis*, and *F. wabuensis* [5]. Among these six species, the classification of *F. wabuensis* is controversial. In Chinese Flora, *F. wabuensis* is a variant of *F. crassicaulis*, while *F. wabuensis* is a variant of *F. unibracteata* in the Chinese Pharmacopoeia. In fact, *F. wabuensis* is related to both *F. crassicaulis* and *F. unibracteata* but more closely to *F. unibracteata* [84]. In addition, there is currently a lack of data on alkaloids in *F. przewalskii*. We compared the types of alkaloids in *F. cirrhosa*, *F. unibracteata*, *F. taipaiensis*, and *F. delavayi* (Table 1), all of which contain imperialine, chuanbeinone, peimine, peiminine, and peimisine (Figures S1, S2 and S4). The geo-authentic bulbus *F. cirrhosae* are well known for good healing effects against chronic cough and asthma [22] in which 21 alkaloids have been identified (Table 1 and Figures S1, S2 and S4–S7). The reported alkaloid types of other base source species of Chuan Beimu are much lower than *F. cirrhosae*. *F. ussuriensis* is used as a substitute for Chuan Beimu in the Northeast region of China [85], which is the only *Fritillaria* growing below 1500 m that contains imperialine.

F. pallidiflora and *F. walujewii* are used as the botanical origins of Yi Beimu in the Chinese Pharmacopoeia. They both contain imperialine, imperialine-3β-D-glucoside, yibeinoside A and peimisine (Table 1 and Figures S2 and S4). A total of 33 alkaloids have been identified in *F. pallidiflora*, while there were 13 alkaloids identified in *F. walujewii*. The method of UPLC-ELSD fingerprint was used for the comprehensive quality evaluation of *F. walujewii* and *F. pallidiflora*, and the fingerprint similarity was $\geq$0.801 [86]. In the fingerprints, the co-peak area of imperialine and imperialine-3β-D-glucoside accounted for 80.32–93.68% of the total peak area.

2.3. Synthesis and Regulation of Alkaloids in Fritillaria

Up to now, the biosynthetic mechanism of various steroidal alkaloids in *Fritillaria* is still not fully understood due to the diverse origins and variable chemical composition. At present, there are two schemes of the biosynthetic pathways of steroidal alkaloids in *Fritillaria* species (Figures 2 and 3). It is currently recognized that the syntheses of various alkaloids in *Fritillaria* may occur via the classical mevalonate (MVA) or 2-methyl-D-erythritol-4-phosphate (MEP) pathways [87]. Along the pathway of reactions catalyzed by specific enzymes in Figure 2, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate

(DMAPP) as 5C-intermediates were synthesized, which resulted in cycloartenol formation and in turn converted to a series of metabolic intermediates, such as farnesyl pyrophosphate (FPP), squalene, and cycloartenol via chair-boat-chair-boat conformational changes, which eventually leads to Cevanine and Jervine types isosteroidal alkaloid biosynthesis by using various modification reactions by CYPs, hydroxysteroid decarboxylase (HSD), isomerase, reductase, methyltransferases, etc. [87–91]. In Figure 2, the biosynthesis of isosteroidal alkaloids was initiated with squalene oxidation and subsequently catalyzed by different forms of (S)-2,3-oxidosqualene cyclase [92], while the frameworks of the Cevanine or Jervine type were formed from cycloartenol by catabolic processes with nitrogen incorporation or hydroxylation reactions [2]. However, in Figure 3, the frameworks of Cevanine type isosteroidal alkaloids and Solanidine and Veratramine type steroidal alkaloids were formed from cholesterol, which was converted into Cevanine type by oxidation, nitrogen incorporation, and multiple reduction reactions [34]. At the same time, through nitrogen incorporation, cholesterol can form Solanidine type and Veratramine type steroidal alkaloids [77].

Previous studies have mainly focused on the isolation, identification, and pharmaceutical activity validation of active ingredients in plants. In recent studies, transcriptome sequencing efforts were often made to reveal the biosynthesis pathways of bioactive compounds for herbal *Fritillaria*. It is worth noting that the synthetic pathway of steroidal alkaloids seems to vary according to different transcriptional analysis for different *Fritillaria* species. In the regenerated bulbs of *F. cirrhosa*, RNA-seq and bioinformatics analysis were performed to study the gene expression profile related to biosynthesis of alkaloids, which showed the MEP pathway was the main route to produce steroidal backbones [89]. *De novo* comparative transcriptome sequencing of bulbs in vivo and in vitro illuminated the positive correlation between a higher expression of biosynthetic pathway genes and a relatively higher accumulation of imperialine in *F. roylei* [90]. However, the MVA pathway was considered as the predominant route for 5C intermediate biosynthesis based on related gene expression and quantitative analysis in *F. roylei*. Subsequently, transcriptome sequencing efforts were made to elucidate isosteroidal alkaloids biosynthesis by creating organ-specific genomic resource of *F. roylei*, which also suggested a primary site of MVA to mediate biosynthesis of isosteroidal alkaloids, while some enzymes involved in the MEP pathway exhibited higher enrichment in leaf tissue [18]. Chemical inhibitors to the rate-limiting enzymes on the two pathways could be effective players to validate the main synthetic pathway of steroidal alkaloids in *Fritillaria*. For instance, the 3-hydroxy-3-methylglutaryl coenzyme A reductase gene (HMGR) was used to remove the feedback regulation of the MVA pathway [93], while the key mutants of squalene oxidase (SQE) in natural evolution of *F. thunbergii*, *F. unibracteata*, and *F. ussuriensis* might play some important roles in differentiating the content of alkaloids [14]. In addition, a repertoire of full-length transcripts of *F. hupehensis* were provided, and flavonoid biosynthesis genes were blasted against those in *Solanum lycopersicum* L. and *Arabidopsis thaliana* (L.) Heynh, which could partially address the weakness caused by the lack of genome [94].

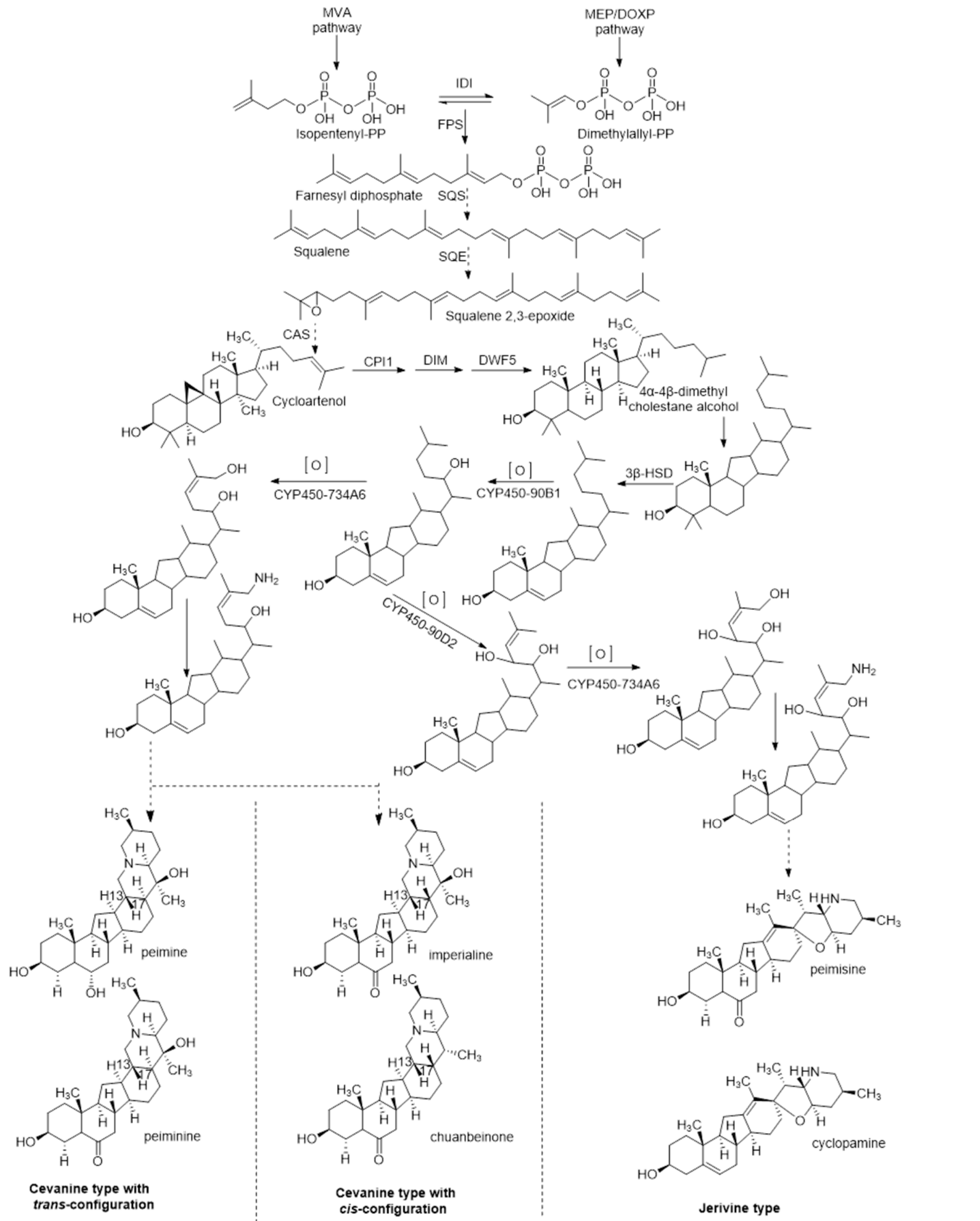


Figure 2. The biosynthetic pathways of Cevanine and Jervine types isosteroidal alkaloids in *Fritillaria* species. Peimine and peiminine are the Cevanine type alkaloids with *trans*-configuration. Imperialine and chuanbeinone are the Cevanine type alkaloids with *cis*-configuration. Peimisine and cyclopamine are the Jervine type alkaloids. IDI: Isopentenyl diphosphate isomerase; FPS: farnesyl diphosphate synthase; SQS: squalene synthase; SQE: squalene oxidase; CAS: cycloartenol synthase; CPI1: cyclopropyl sterol isomerase1; DIM: delta (24)-sterol reductase; DWF5: 7-dehydrocholesterol reductase; 3β-HSD: 3β-hydroxysteroid decarboxylase; CYP450-90B1: C-22 hydroxylase.

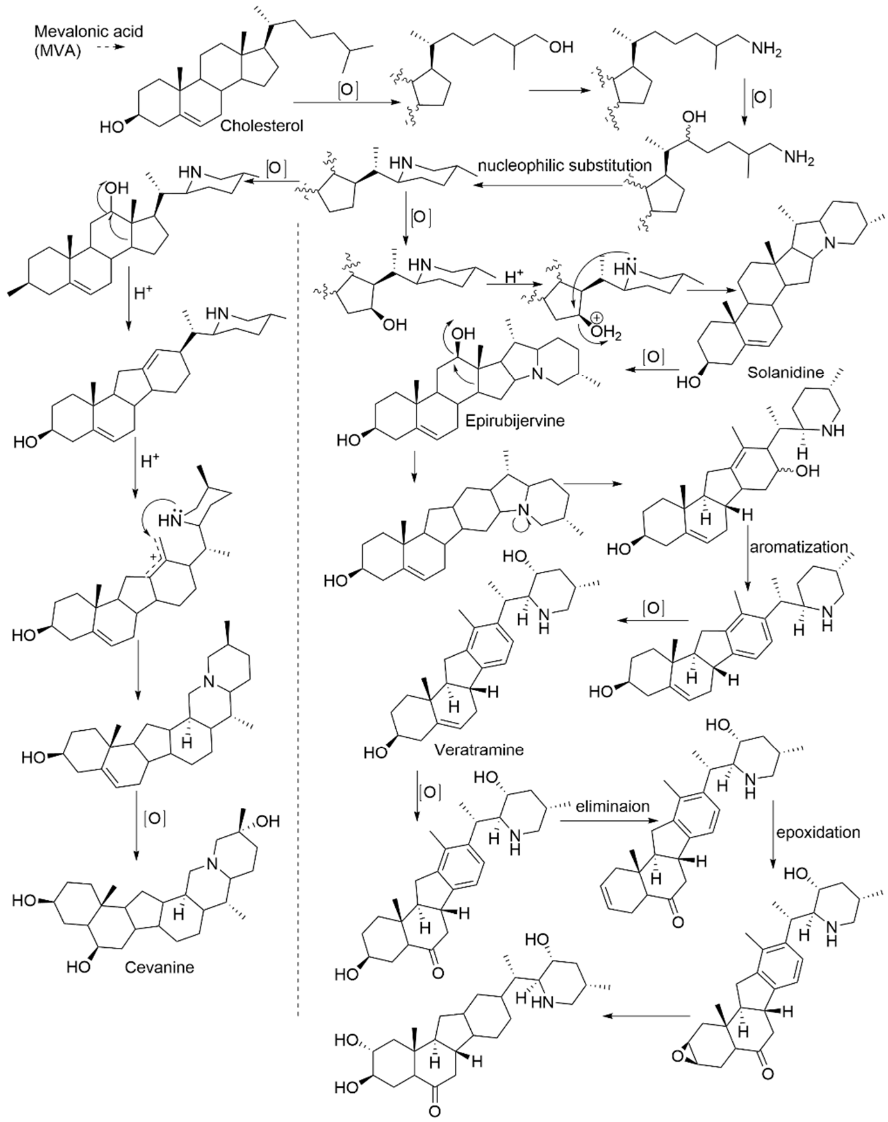


Figure 3. The biosynthetic pathways of Cevanine type isosteroidal alkaloids and Solanidine and Veratramine type steroidal alkaloids in *Fritillaria* species.

3. Taxonomy of *Fritillaria* Species

Some estimates suggest that there are approximately 140 *Fritillaria* species in the world [95]. However, by searching some mainstream plant taxonomic databases (eFloras (http://www.efloras.org/ (accessed on 20 July 2022)), Flora of China (http://www.iplant.cn/ (accessed on 20 July 2022)), Fritillaria (Pacific Bulb Society) (https://www.pacificbulbsociety.org/pbswiki/index.php/Fritillaria (accessed on 20 July 2022)), and World Checklist of Selected Plant Families (WCSP) (https://wcsp.science.kew.org/ (accessed on 20 July 2022)), we found a total of 158 *Fritillaria* species in the world (Tables S1 and S2), including 91 species in Asia (Centered in the Himalayas and Qinghai-Tibet Plateau), 41 species in Europe (Centered in Greece), and 21 species in North America (Centered in California), with the rest being randomly distributed. In recent years, several new *Fritillaria* species have been identified but they have yet to be studied [52,96].

3.1. The Taxonomy Based on Classical Methods

Fritillaria species can be classified and identified by classical methods based on their morphological features. In principle, various morphological parameters, including but not limited to stem height, leaf shape and phyllotaxis, inflorescence characteristics, pollen, capsule, bulbs, and starch grains in bulbs may be selected for plant identification [83,97].

Of these identifying morphological features, pollen and starch grains in bulbs may provide a higher resolution for *Fritillaria* identification [97]. *F. thunbergii*, *F. cirrhosa*, *F. ussuriensis*, *F. hupehensis*, and *F. pallidiflora* were taxonomically identified on starch grains [97]. The short diameter of a bulb with less intra-specific but more inter-specific variations could be used as one of the key indicators for identification of bulbus *Fritillariae* [83]. As a traditional, intuitive, and convenient method, however, morphological features alone are not sufficient to accurately categorize *Fritillaria* species.

Elevation usually interacts with temperature and light intensity, which has a significant influence on metabolite accumulation in underground bulbs [98]. *Fritillaria* species can be roughly classified into three groups according to distributed elevations (Table 1). Group 1 *Fritillaria* species, which are as the botanical origins of Chuan Beimu, are mainly distributed at 2700–4000 m. Group 3 *Fritillaria* species are mainly distributed below 1500 m. In addition, Group 2 *Fritillaria* species are mainly distributed between 1500–2700 m. By searching mainstream plant taxonomic databases (eFloras (http://www.efloras.org/ (accessed on 20 July 2022)), Flora of China (http://www.iplant.cn/ (accessed on 20 July 2022)), the correlation between elevation and morphological features of *Fritillaria* species were summarized. The elevation and biomass are correlated. As shown in Figure 4C and Table 1 (Group 1), these *Fritillaria* species growing between 2700–4000 m are generally 15–50 cm in length and usually open a single flower. While those species growing below 1500 m are normally taller than 50 cm and will blossom a few flowers to form a racemose or umbellate inflorescence (Figure 4B and Group 3 in Table 1). However, morphological features are vulnerable to geographic, environmental, and climatic influences, and they could only be used as a classification aid.

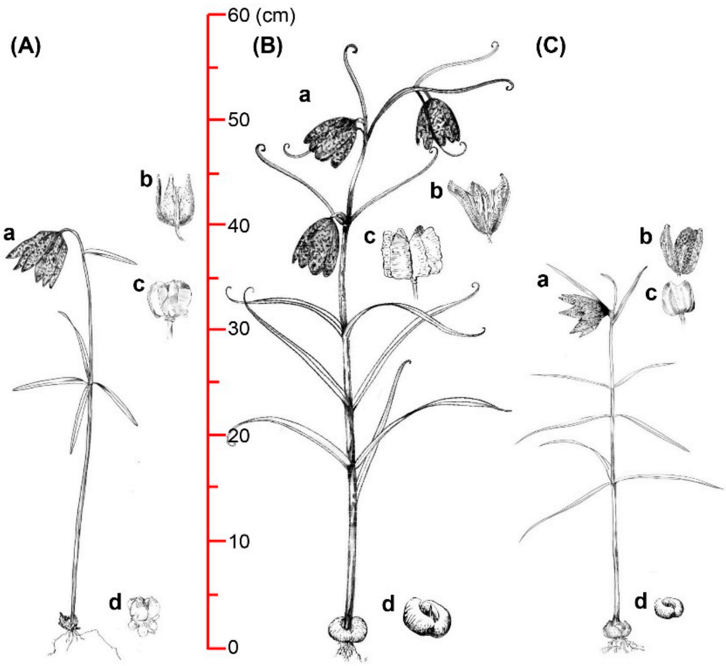
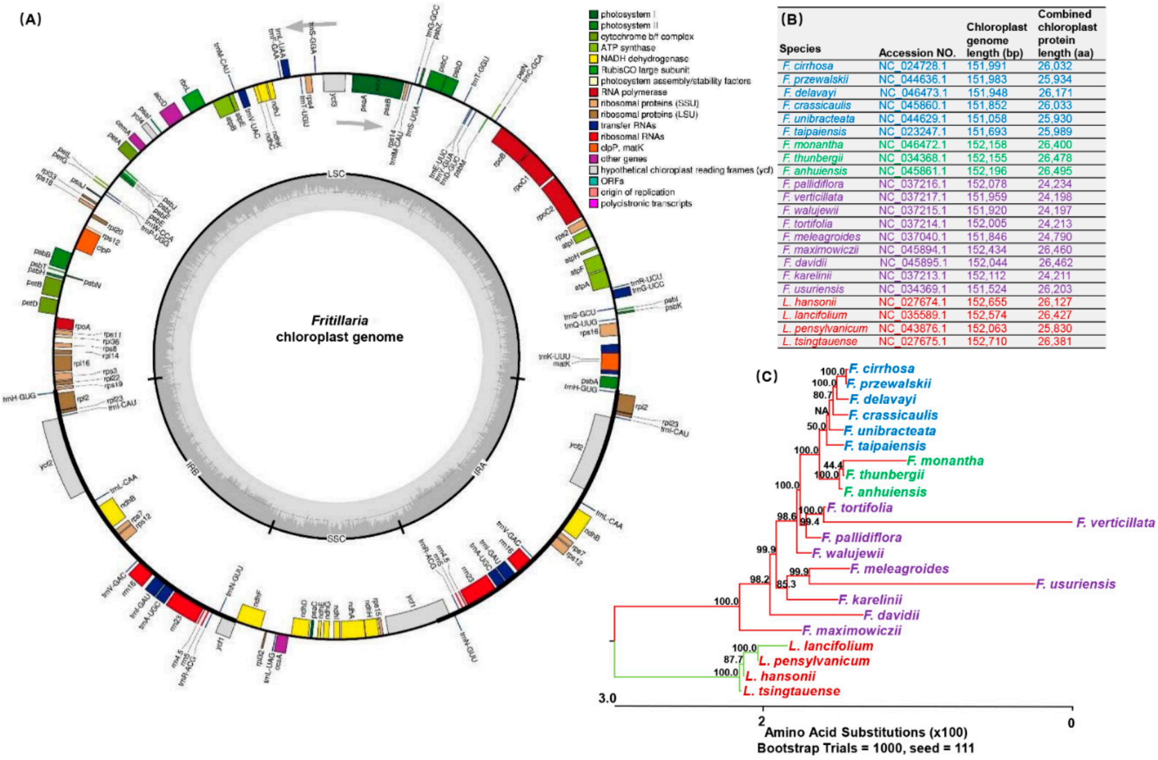


Figure 4. The morphological ideograms of the representative *Fritillaria* species in three altitude groups. (**A**). The morphological features of *F. maximowiczii* Freyn growing distributed between 1500–2700 m in Group 2. a. The plant of *F. maximowiczii*. b. The flower of *F. maximowiczii*. c. The capsule of *F. maximowiczii*. d. The bulb of *F. maximowiczii*. (**B**). The morphological features of *F. thunbergii* growing below 1500 m in Group 3. a. The plant of *F. thunbergii*. b. The flower of *F. thunbergii*. c. The capsule of *F. thunbergii*. d. The bulb of *F. thunbergii*. (**C**). The morphological features of *F. cirrhosae* growing between 2700–4000 m in Group 1. a. The plant of *F. cirrhosae*. b. The flower of *F. cirrhosae*. c. The capsule of *F. cirrhosae*. d. The bulb of *F. cirrhosae*.

3.2. Molecular Decoding- and Phylogenetic Analysis-Based Taxonomy

DNA barcoding is a widely used tool for rapidly identifying plant species, but none of the available loci works across all species [99,100]. Both ITS1 and ITS2, which are internal regions between 18S and 5.8S and 5.8S and 28S, respectively, are also used as molecular markers for phylogenetic relationships [98]. Due to low rates of nucleotide substitutions, lack of recombination, and restriction of uni-parental inheritance, the chloroplast genome (cp genome) is more conserved compared to nuclear and mitochondrial genomes [101–103]. A variety of intergenic spacers, genes in the cp genome, and the entire cp genome of *Fritillaria* have been utilized to establish a phylogenetic tree. However, phylogenetic analysis based on some barcodes generally demonstrated low resolution, especially for the deep phylogenetic relationships with short internodes and fast rates [85]. Based on the aligned assembly of tree chloroplast markers (*matK*, *rbcL*, *rpl16*) and nuclear ITS, phylogenetic relationships of *Liliaceae* species (including *Fritillaria*) were analyzed to indicate that *Lilium* was nested within *Fritillaria* to be paraphyletic and partitioned into two monophyletic clades, but these results were not supported by nuclear ITS data [104]. The ITS1, ITS2, ITS1 + ITS2, and cp phylogenetic trees were constructed, and the evolutionary distances based on ITS1 + ITS2 and overlapping extent showed a positive correlation with a relatively higher accuracy and lower *p*-values [98]. The phylogenetic relationship of *Fritillaria* species was also analyzed based on 64 single-copy genes and the whole chloroplast genomes of 8 subgenera further confirmed the species to be monophyletic, except for the polyphyletic subgenus *Fritillaria* [105]. The reported results revealed the genus *Fritillaria* to be a sister to *Lilium*. Furthermore, the phylogenetic tree of 7 *Fritillaria* species with combined nucleotides of 74 common protein-coding genes was constructed, which provided a highly supportive bootstrap [106]. The phylogenetic tree based on an entire cp genome also showed high resolution for *Fritillaria* species with individuals of each species in a monophyletic clade [103].

In this review, the phylogeny of 18 medicinal *Fritillaria* species was compared to the combined amino acid sequences of chloroplasts from 74 common protein-coding genes, and 4 *Lililum* species of *Liliaceae* family set as the outgroups (Figure 5), and the phylogenetic relationship was relatively consistent with the discovery based on 64 single-copy genes and entire cp genomes [103,105]. It was interesting that these medicinal *Fritillaria* species could be roughly divided into three groups, and the phylogenetic relationships of these species, except for *F. ussuriensis*, coincided with the three groups classified by the elevations (Table 1). The groups included *F. cirrhosa*, *F. przewalskii*, *F. delavayi*, *F. crassicaulis*, *F. unibracteata*, and *F. taipaiensis*, all of which showed homologous relationships, grew at the same elevation and displayed morphological similarity. These were the main sources of Chuan Beimu (Group 1 in Table 1 and Figure 4C). *F. thunbergii*, *F. monantha*, and *F. anhuiensis* grew below 1500 m, and they also showed a high degree of homology. Moreover, these mainly contain 5α-cevanine isosteroidal alkaloids with *trans*-configuration (Group 3 in Table 1 and Figure 4B). The above results indicated that phylogenetic analysis clarified the evolutionary relationships of species, and they serve as an important parameter for the classification and the identification of species. Future phylogenomic studies require the barcodes with higher resolution and more samples with extensive representation of taxonomy.



Species	Accession NO.	Chloroplast genome length (bp)	Combined chloroplast protein length (aa)
F. cirrhosa	NC_024728.1	151,991	26,032
F. przewalskii	NC_044636.1	151,983	25,934
F. delavayi	NC_046473.1	151,948	26,171
F. crassicaulis	NC_045860.1	151,852	26,033
F. unibracteata	NC_044629.1	151,058	25,930
F. taipaiensis	NC_023247.1	151,693	25,989
F. monantha	NC_046472.1	152,158	26,400
F. thunbergii	NC_034368.1	152,155	26,478
F. anhuiensis	NC_045861.1	152,196	26,495
F. pallidiflora	NC_037216.1	152,078	24,234
F. verticillata	NC_037217.1	151,959	24,198
F. walujewii	NC_037215.1	151,920	24,197
F. tortifolia	NC_037214.1	152,005	24,213
F. meleagroides	NC_037040.1	151,846	24,790
F. maximowiczii	NC_045894.1	152,434	26,460
F. davidii	NC_045895.1	152,044	26,462
F. karelinii	NC_037213.1	152,112	24,211
F. usuriensis	NC_034369.1	151,524	26,203
L. hansonii	NC_027674.1	152,655	26,127
L. lancifolium	NC_035589.1	152,574	26,427
L. pensylvanicum	NC_043876.1	152,063	25,830
L. tsingtauense	NC_027675.1	152,710	26,381

Figure 5. The phylogeny of major medicinal *Fritillaria* species based on the combined chloroplast protein sequences. (**A**). The chloroplast genome map of major medicinal *Fritillaria.* (**B**)The combined chloroplast protein length of major medicinal *Fritillaria* species. (**C**). The phylogeny of major medicinal *Fritillaria* species based on the 74 combined chloroplast protein sequences. Major medicinal *Fritillaria* species could divide into three groups based on the evolutionary tree.

4. Domestication and Culture of *Fritillaria* Species

With the advancement in analytical chemistry and pharmacology as well as available scientific and experimental research, the pharmacological effects of *Fritillaria* have been gradually and widely recognized in the world, and especially the successful application in COVID-19 treatment and prevention [107,108], which has greatly increased the market demand for herbal *Fritillaria*. Currently, more than 1500 medicines patented in China contain raw ingredients originated from the bulb or other tissues of medicinal *Fritillaria* [12]. Unfortunately, the huge economic benefits from *Fritillaria* species were generated at the cost of their overexploitation. Up until January 2022, 20 *Fritillaria* species have been listed in the IUCN Red List of Threatened Species (https://www.iucnredlist.org/ (accessed on 20 July 2022)) (Table S2). To balance economic requirements and ecological protection, domestication and resource conservation of *Fritillaria* species must go hand in hand.

4.1. Artificial Cultivation of Fritillaria Species

Artificial cultivation is an effective way to protect wild resources and relieve the contradiction between supply and demand in the *Fritillaria* industry. Up to now, several *Fritillaria* species, including *F. thunbergii*, *F. monantha*, *F. anhuiensis*, *F. ussuriensis*, *F. cirrhosa*, *F. unibracteata*, *F. taipaiensis*, and *F. delavayi*, have been successfully cultivated artificially [1,94,109–111]. The reproduction of bulbs was the main method to domesticate *Fritillaria* species [109]. Since the growth period of bulb reproduction usually takes approximately 100 days–3 years [4], the bulbs would be used as seeds in advance. While reproductive cycle by seed generally takes more than five years in *Fritillaria* species [93].

As a typical representative, the bulb of *F. thunbergii* has been commercially reproduced in China over the last 700 years, and the reproduction technology was adopted approximately 300 years ago [112]. *F. thunbergii* as a member of the herbal drugs "Zhebawei" is widely cultivated in the south-eastern coastal, south-central, and eastern areas of China, mostly in the provinces of Zhejiang (Figure 6), Jiangsu, Anhui, Jiangxi, and Hunan. In Xiangshan county of Ningbo in Zhejiang province, the wild *F. thunbergii* was initially domesticated in 1600–1644 AD [111]. Between 1488 and 1722 AD in Qing Dynasty, the seeds of *F. thunbergii* were spread from Xiangshan county to Zhangshui town of Ningbo. In Panan County of Jinhua city Zhejiang province, *F. thunbergii* var. *chekiangensis* was cultivated in the late Qing Dynasty, and *F. thunbergii* was introduced and cultivated in the 1970s [113]. Both the whole and the partial bulb of *F. thunbergii* germinate, but the morphological features of the plants and bulbs from the two germination ways would be different. While artificial cultivation continues to grow year on year, the wild resources of *F. thunbergii* have gradually vanished due to a lack of strict protection and scientific management. According to the local herb farmers, nowadays the wild *F. thunbergii* can occasionally be found only in the Temmoku Mountain. In recent years, the imitating wild cultivation of *F. thunbergii* has become more popular (Figure 6B), which could slow down *Fritillaria* species degradation. The comparison between artificial cultivation and imitating wild cultivation will be a research direction in the future.

Figure 6. Artificial and imitating wild cultivation of *F. thunbergii*. (**A**). The large-scale artificial cultivation of *F. thunbergii* in Zhangshui town of Ningbo in Zhejiang Province of China. (**B**). The imitating wild cultivation of *F. thunbergii* in Siming Mountain in Zhejiang Province of China. (**C**). The *F. thunbergii* Flos. (**D**). The bulb of *F. thunbergii* by artificial cultivation. Bar: 1 cm. (**E**). The bulb of *F. thunbergii* by the imitating wild cultivation. Bar: 1 cm.

Moreover, *F. pallidiflora* native to Xinjiang Uygur Autonomous region have been domesticated in several regions of Inner Mongolia, Gansu, Shaanxi, Henan, and Shandong provinces in China since 1965 [114]. *F. hupehensis* has been cultivated in main production areas in Enshi City of Hubei Province for more than 200 years and been used as Chuan Beimu before being recorded in Chinese Pharmacopoeia [115,116]. *F. ussuriensis* naturally growing in the Northeast region of China has been cultivated for more than 100 years; and, until 1984, it had been domesticated in Shandong, Hebei, Jiangsu, Shaanxi, Henan, and Jiangxi provinces [117,118].

Currently, the species of *Fritillaria* growing at lower altitudes can be cultivated by bulb reproduction, but species growing at higher altitudes still rely mainly on gathering wild resources [119]. Bulb reproduction would firstly consume bulbs as seeds and thus cause some economic losses to famers. Many *Fritillaria* species are now protected and cannot be collected without authorization. In addition, the quality of *Fritillaria* species cultivated by bulb reproduction usually does not meet the standards of the morphology or the contents of active constituents. Low propagation rate limited the extension of bulb reproduction. It is also important to know that species degeneration and serious diseases could be caused by multiple asexual reproductions.

4.2. In Vitro Cultivation of Fritillaria Species

In recent years, developing in vitro cultivation techniques of medicinal plants has been becoming a research hotspot [17,89,120]. The tissue culture of *Fritillaria* focused on the induction of the regenerative bulb and the polyploid [93,109]. Since the low survival rate of plantlets in test tubes severely limited large-scale cultivation in the field, in vitro bulb regeneration has become an efficient strategy to alleviate the excessive demand for bulbus *Fritillariae*. In addition, regenerated bulbs are thought to accumulate more alkaloids than wild bulbs [89]. The proper hormone composition and concentration, light, and temperature were critical to regenerate the bulbs. So far, the methods and techniques for the tissue culture of *F. thunbergii* (Figure 7), *F. cirrhosa*, *F. unibracteata*, *F. anhuiensis*, *F. taipaiensis*, *F. ussuriensis*, and *F. pallidiflora* have been established [89,109,110,120,121]. The *Fritillaria* bulbs regenerated via in vitro cultivation may promise to reduce the market pressure due to overexploitation of wild resources. However, the difference in morphological features and phytochemical profile between the regenerated bulbs and the wild bulbs may hinder the marketization of in vitro bulb regeneration.

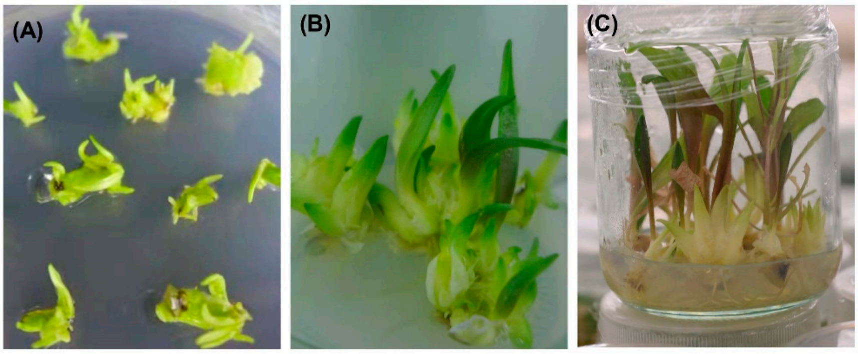


Figure 7. The tissue culture of *F. thunbergii*. (**A**). Callus induced from bulb section of *F. thunbergii*. (**B**). The redifferentiation of callus of *F. thunbergii*. (**C**). Tissue culture plantlets of *F. thunbergii* induced rooting.

The appropriate conditions for the dormant termination and germination of seeds and the effective methods of bulb propagation have been investigated and simulated for artificial cultivation of *Fritillaria* species. Therefore, an appropriate combination of asexual (tissue culture and bulbs reproduction) and sexual reproduction (artificial cultivation by seed reproduction) may provide an effective and most suitable way for *Fritillaria* domestication and cultivation. For the effective protection and sustainable utilization of valuable *Fritillaria* species, there is an urgent need to meticulously survey the growth environment, overcome the difficulties on genetic breeding and domestication, and develop scientific methods of artificial or imitating wild cultivation and tissue culture. For *Fritillaria* species growing at 2700–4000 m, the natural fostering system may also provide an effective approach to vigorously protect and sustainably use them.

4.3. The Management of Fritillaria Diseases

Continuous cropping in key *Fritillaria* producing has resulted in a decline in soil organic matter content, degradation of soil structure, nutritional imbalance, pathogen accumulation, and a serious incidence of plant disease in recent years [122]. Pathogen-caused diseases are the primary cause of *Fritillaria* output declines, and four prevalent diseases in *Fritillaria* species are sclerotinia infections, root rot, gray mold, and rust [123]. Field management and chemical and biological controls were mostly used to manage diseases of medicinal *Fritillaria* [124]. The foundation for lowering the prevalence of diseases is field management, and it is typically necessary to get rid of pathogen spores, mycelium, and other bacteria that cause soil-borne diseases, pests, and their parasite eggs [125]. Reasonable fertilization is also essential to enhance the disease resistance of *Fritillaria* [126,127]. Applying the right quantity of biochar can raise the production of *F. thunbergii*, decrease the occurrence of fusarium wilt, and improve the alkaloid content of *Fritillaria* by lowering the soil's bulk density, and raising the pH, total nitrogen, and accessible potassium [122]. Carbendazim and Hymexazol are two chemical pesticides that are useful in preventing and controlling *Fritillaria* diseases, but they also carry a risk of pesticide residues [128]. The control of *Fritillaria* diseases has greatly benefited through the use of biological pesticides in recent years. Amistar SC and Junkeduke AS have a 10.5–15.7% greater control efficacy than chemical pesticides for *F. ussuriensis* rust [129]. There are reports and applications of the biocontrol bacteria for sclerotinia diseases, root rot, and gray mold in biological controls. The bacteriostasis rate of Trichoderma virens T43 and its fermented extract against the sclerotinia disease for *F. ussuriensis* was up to 60% [130]. At present, there are few studies on the biological control of *Fritillaria* rust caused by *Uromyces lilii* [124].

5. Conclusions and Discussion

Fritillaria, as a famous traditional Chinese herb with a slightly cool nature and low toxicity, possesses good efficacy of reducing fever as well as moistening the lungs and dispelling fire in the human body. There are 158 *Fritillaria* species worldwide, 33 of which have reported therapeutic efficacy, and 20 of non-reported ones were listed in the IUCN Red List of Threatened Species. Due to the large number and complexed provenance, the establishment of high-resolution, effective, convenient, and reliable methods and techniques is of great significance for identification of various *Fritillaria* species. Herein, we have summarized the taxonomy of *Fritillaria* species based on classical methods and phylogenetic analysis. All existing data and analytic results indicate that the geographical environment, especially growth elevation may have an important influence on the phytochemical components and morphological features of *Fritillaria* during the evolutionary process.

At present, there are more than 100 alkaloids that have been isolated from *Fritillaria* species (Table 1 and Figures S1–S7). Although it is difficult to completely distinguish the type of chemicals by distributed elevation, isosteroidal and steroidal alkaloids, which are responsible for the pharmacological activities of bulbus *Fritillariae*, are significantly different between three major categories. *Fritillaria* species are the sources of Chuan Beimu, and they mainly contain more 5α-cevanine isosteroidal alkaloids with *cis*-configuration. In contrast, species growing below 1500 m mainly contain more the *trans*-configuration isosteroidal alkaloids. The biosynthetic mechanism of various alkaloids in *Fritillaria* is still not fully understood due to the diverse origins and variable chemical composition. According to the available literature, there are two schemes of the biosynthetic pathways of *Fritillaria* alkaloids with different frameworks and catalytic reactions. In Figure 2, the biosynthesis of Cevanine or Jervine type isosteroidal alkaloid were formed from cycloartenol by catabolic processes with nitrogen incorporation or hydroxylation reactions (Figure 2). However, in Figure 3, the frameworks of Cevanine type isosteroidal alkaloids and Solanidine and Veratramine type steroidal alkaloids were formed from cholesterol (Figure 3). Yet, the synthetic pathway of alkaloids seems to vary according to high-throughput omics data for different *Fritillaria* species. The discovery of intermediates in biosynthetic pathways may be the strongest scientific evidence for both schemes. The transcripts of alkaloids biosynthesis genes compared to those in pattern species may help to compensate for the lack of *Fritillaria* genome.

Artificial or in vitro cultivations are effective strategies for balancing economical requirements and ecological protection. *Fritillaria* species growing at lower altitudes can be cultivated by bulb reproduction, but species growing at higher altitudes still rely mainly on gathering a large number of wild resources. The bulbus of Chuan Beimu and Zhe Beimu are commonly recognized to be excellent in quality, but their costs are significantly different because the latter can be cultivated by domestication. *Fritillaria* species used as the botanical origins of Chuan Beimu are mainly distributed in the alpine areas of the Himalayan-Hengduan Mountains with an altitude of 2700–4000 m (Table 1). The high distributed elevation makes Chuan Beimu susceptible not only to climate change, but also to human activities. Chuan Beimu as the alpine plants are expected to be sensitive to anthropogenic climate change because of their cold-adapted, which have been classified as endangered species under the third level of protection in regulations issued by the

Chinese government since 1980s [13,131]. Detecting the environmental factors in the geographical distribution are critical issues for the artificial cultivation of Chuan Beimu, which is considered to be an important way to resolve the current contradiction between resource protection and utilization [98]. There were reported about the artificial cultivation of Chuan Beimu in 1985 [132]. In 2017, the area for artificial cultivation of *F. cirrhosae* were larger than 400 hm^2 and productivity was higher than 180 t [1], which still could not meet the market demand of Chuan Beimu, resulting in its high price and the overexploitation of wild resources. In contrast, the area for artificial cultivation of *F. thunbergii* reached 4.1×10^3 hm^2 and productivity was higher than 1.258×10^4 t in Zhejiang Province, China in 2019 [133]. Excess inventory leads to an annual decline in the price of Zhe Beimu. In addition, most *Fritillaria* species in the world (Tables S1 and S2) have not been medicinally studied and exploited. The exploitation of excellent *Fritillaria* species based on resource investigation, pharmacological application, quality evaluation, and commodity circulation may be another important way to resolve the imbalance in the *Fritillaria* industry. Meanwhile, integration of asexual tissue culture and bulb reproduction with sexual artificial or imitated wild cultivation may create a very promising and effective way to maintain sustainable development of *Fritillaria* in industry.

6. Data Collection

Fritillaria species data were searched and collected from a number of scientific databases, including: eFloras (http://www.efloras.org/ (accessed on 20 July 2022)), Flora of China (http://www.iplant.cn/ (accessed on 20 July 2022)), Fritillaria | Pacific Bulb Society (https://www.pacificbulbsociety.org/pbswiki/index.php/Fritillaria/ (accessed on 20 July 2022)),World Checklist of Selected Plant Families (WCSP) (https://wcsp.science.kew.org/ (accessed on 20 July 2022)), Web of Science (http://apps.webofknowledge.com/ (accessed on 20 July 2022)), Scopus (https://www.scopus.com/ (accessed on 20 July 2022)), PubMed (https://pubmed.ncbi.nlm.nih.gov/about/ (accessed on 20 July 2022)), Google (https://google.com/ (accessed on 20 July 2022)), Google Scholar (http://scholar.google.com/ (accessed on 20 July 2022)), Sci-Finder (http://scifinder.cas.org/ (accessed on 20 July 2022)), Science Direct (https://www.sciencedirect.com/ (accessed on 20 July 2022)), CNKI (www.cnki.net/ (accessed on 20 July 2022)), Wanfang (www.new.wanfangdata.com.cn/index.html/ (accessed on 20 July 2022)), and the IUCN Red List of Threatened Species (https://www.iucnredlist.org/ (accessed on 20 July 2022)). Further, additional information on *Fritillaria* was also available through traditional Chinese medicinal books, local chronicles, and botanical books.

A bootstrap neighbor-joining phylogenetic tree of *Fritillaria* based on the combined amino acid sequences of chloroplast 74 common protein-coding genes was constructed using the MegAlign Clustal W method with the sequences of *Lililum* as the anchors. The detailed sequence data here can be found in NCBI (https://www.ncbi.nlm.nih.gov/ (accessed on 20 July 2022)) databases, and the accession numbers are listed in Figure 5.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12081844/s1, Figure S1: The chemical structures of 5α-Cevanine isosteroidal alkaloids with *trans*-configuration.; Figure S2: The chemical structures of 5α-Cevanine isosteroidal alkaloids with *cis*-configuration; Figure S3: The chemical structures of other 5α-Cevanine isosteroidal alkaloids; Figure S4: The chemical structures of Jervine isosteroidal alkaloids; Figure S5: The chemical structures of Veratramine isosteroidal alkaloids; Figure S6: The chemical structures of Verazine steroidal alkaloids; Figure S7: The chemical structures of Solanidine steroidal alkaloids; Table S1: *Fritillaria* species with medicinal activity in the world; Table S2: Other *Fritillaria* species in the world (*Fritillaria* species with E as superscript were endangered). References [134–151] are cited in Supplementary Materials.

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Z.W. (Zhonghua Wang); supervision, Q.W. (Qiang Wang); project administration, Z.W. (Zhi'an Wang); funding acquisition, Q.W. (Qiang Wang). A.Q. and Q.W. (Qingfei Wu) contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

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