# Molecular phylogeny and character evolution of *Fritillaria* subgenus *Liliorhiza*, Liliaceae

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## Abstract

Phylogenetic relationships within *Fritillaria* subgenus *Liliorhiza* have not been resolved. Ronsted et al. (2005) determined the circumscription of *Fritillaria*, but included only 12 of the approximately 20-25 species in the subgenus. Recent classifications based on morphology (Santana, 1984; Rix, 2001) are in conflict and may be resolved by a comprehensive molecular study. My study aims to determine phylogenetic relationships of as many described taxa in the group as possible, to evaluate taxonomic classifications (including questionable species and varieties), and to explore morphological character evolution. To accomplish these goals, I have initiated acquisition of specimens and sequences from two chloroplast regions and nuclear ribosomal ITS and ETS. Preliminary results of phylogenetic studies using Bayesian phylogenetic inference, species tree estimation, and ancestral character state reconstruction, will be presented. Previously hypothesized subdivisions based on morphology are partially supported in my molecular analysis, but a complete molecular data set must be obtained before any definitive reclassification can be made. One moderately supported clade (pp=0.73) corresponds with Subsection *Affines* (Santana, 1984) and Section B1 (Rix, 2001) with a few exceptions. Another clade, though not supported, corresponds with Subsection *Liliorhiza* (Santana, 1984) and Section B2 (Rix, 2001). With regard to character evolution, rice-grain bulbils appear to be the ancestral state for the group, and have been lost once in a weakly supported clade of four species; whorled leaf arrangement appears to be the ancestral state for the group, and appears to have changed to alternate leaf arrangement a minimum of three times, with one reversal.

## Objectives

1. Construct a molecular phylogeny of *Fritillaria* subgenus *Liliorhiza*.
2. Evaluate taxonomic classifications within the group.
3. Explore evolutionary history using ancestral character state reconstruction.

## Methods and Materials

### Molecular Data
- Sequences of matK, rpl16, and ITS, from 13 taxa of *Fritillaria* (Ronsted et al. 2005) were obtained from GenBank. Note: *F. phaeanthera* is considered synonymous with *F. affinis* in the Jepson Manual. *F. eastwoodiae* and *F. striata* lack matK sequences.
- New sequences of rpl16 and ITS from 8 taxa, including one duplicate of *F. affinis*, a suspected specimen of *F. affinis* "tristulis", and an unidentified specimen (*F.* "ritteri") from Monterey County, were obtained using standard lab protocols.
- Amplification and sequencing of the matK region is currently in progress.
- A *Fritillaria*-specific ETS primer will be designed using the protocol of Baldwin and Markos (1998).
- All taxa to be included in the study are listed in Table 1.

### Phylogenetic analysis
- Species tree estimation performed using *BEAST (Drummond and Rambaut, 2007).
- Analyses were run for 100 million generations.
- Implemented a relaxed lognormal clock (Drummond et al. 2006).
- Nucleotide substitution models selected using jModelTest (Posada, 2008).
- Maximum clade credibility tree obtained using TreeAnnotator v1.5.4.

### Ancestral Character State Reconstruction
Character states for bulb morphology and leaf arrangement came from Santana (1984), the Jepson Manual, the Flora of North America, original species descriptions, and personal observations. These were coded within Mesquite v2.72 (Maddison and Maddison, 2009), traced over the maximum clade credibility tree, and analyzed under parsimony optimized ancestral state reconstruction (Figure 2).

## Preliminary Results

### Phylogenetic Analysis
The same relationships among species studied by Ronsted et al. (2005) are recovered with the inclusion of additional taxa, but confidence values (posterior probabilities) are low for several nodes. *F. striata* and *F. agrestis* are closely related (pp=0.87), which agrees with Santana (1984) but not Rix (2001). *F. viridea* and *F. falcata* are closely related (pp=0.79), which disagrees with both morphological classifications. The low support values for many nodes may be due to the lack of matK sequences for 10 taxa and that only one individual per taxon is included. Coalescent methods like *BEAST are designed to include several individuals per taxon and are known to be sensitive to missing sequences. Conclusions about the phylogeny of subgenus *Liliorhiza* cannot be drawn until more taxa, represented by multiple individuals and sequences from all gene regions, are included in the data set.

## Preliminary Results (Cont.)

### Ancestral Character State Reconstruction
Rice-grain bulbils are unique within *Fritillaria* to subgenus *Liliorhiza*, with the exception of *F. davidii*, which has been hypothesized to have been derived independently (Ronsted et al. 2005). They appear to be the ancestral state, and have been lost once in a weakly supported clade of four species. These bulbils detach from the mother bulb to become new individuals, thereby enhancing a population's ability to expand asexually. The adaptive significance of losing this feature is not yet known. Whorled leaf arrangement appears to be the ancestral state and has changed to alternate leaf arrangement a minimum of three times with one reversal. The maximum clade credibility tree has many unsupported nodes, and not all taxa have been sampled. Once all taxa have been examined, I will be able to draw more specific conclusions about the evolution of these characters.

## Future Work

I will continue collecting specimens this spring, and obtaining sequences to complete my molecular data set (Table 1). I will focus my collections to include multiple individuals from each taxon, particularly taxa with questionable taxonomic validity such as *F. affinis* "tristulis" and *F.* "roderickii". These may be of conservation concern if they are indeed valid taxa. A full complement of sequences for each taxa will improve my species tree estimations, since these can then be analyzed under the coalescent model. I also intend to add more morphological characters to perform further ancestral character state reconstructions, and will look for patterns in the biogeographic distributions of each taxon.

Distribution of North American *Fritillaria* from the Flora of North America

*F. affinis* (San Luis Obispo Co.)

*F. affinis* "tristulis" (Marin Co.)

*F. biflora* var. *biflora* (San Luis Obispo Co.)

*F. recurva* (Colusa Co.)

*F. ojaiensis* (Santa Barbara Co.)

*F. glauca* (Glenn Co.)

*F. agrestis* (San Luis Obispo Co.)

*F. purdyi* (Glenn Co.)

"Rice-grain" bulbils

Bulbils absent

Leaves whorled

Leaves Alternate

### Bulb Morphology

### Leaf Arrangement

Table 1. Recent morphological classifications | Taxa included in molecular studies

| Santana (1984) | Rix (2001) | Ronsted et al. (2005) | Current Study |
|---|---|---|---|
| **Subsection *Affines*** | **Section B1** | **Section B1** | **Current Study** |
| Series *Affines* | *F. affinis* | *F. affinis* | *F. affinis* |
| *F. affinis* | *F. affinis* var. *tristulis* | *F. agrestis* | *F. affinis* "tristulis" |
| *F. brandegeei* | *F. anhuiensis* | *F. camschatcensis* | *F. agrestis* |
| *F. eastwoodiae* | *F. atropurpurea* | *F. eastwoodiae* | *F. atropurpurea* |
| *F. glauca* | *F. brandegeei* | *F. falcata* | *F. biflora var. biflora* |
| *F. micrantha* | *F. camschatcensis* | *F. glauca* | *F. camschatcensis* |
| *F. recurva* | *F. dagana* | *F. maximowiczii* | *F. eastwoodiae* |
| *F. viridea* | *F. eastwoodiae* | *F. micrantha* | *F. falcata* |
| Series *Falcatae* | *F. gentneri* | *F. phaeanthera* | *F. gentneri* |
| *F. atropurpurea* | *F. maximowiczii* | *F. pudica* | *F. glauca* |
| *F. falcata* | *F. micrantha* | *F. recurva* | *F. maximowiczii* |
| *F. ojaiensis* | *F. pinetorum* | *F. striata* | *F. micrantha* |
| *F. pinetorum* | *F. recurva* | | *F. ojaiensis* |
| **Subsection *Liliorhiza*** | *F. shuchengensis* | **Taxa not yet sampled** | *F. phaeanthera* |
| *F. agrestis* | *F. wuyangensis* | *F. biflora var. ineziana* | *F. pinetorum* |
| *F. biflora* | **Section B2** | *F. brandegeei* | *F. pudica* |
| *F. camschatcensis* | *F. agrestis* | *F. liliacea* | *F. purdyi* |
| *F. liliacea* | *F. biflora ssp. biflora* | *F. pluriflora* | *F. recurva* |
| *F. purdyi* | *F. biflora ssp. ineziana* | *F.* "roderickii" | *F. striata* |
| *F. pluriflora* | *F. falcata* | | *F. viridea* |
| *F. falcata* | *F. glauca* | | ~*F.* "ritteri" |
| *F. roderickii* | *F. grayana*("roderickii") | | |
| *F. striata* | *F. liliacea* | | |
| **Subsection *Pudicae*** | *F. pluriflora* | | |
| *F. pudica* | *F. purdyi* | | |
| | **Section B3** | | |
| | *F. pudica* | | |
| | **Section B** | | |
| | *F. striata* | | |
| | *F. viridea* | | |

\* treated as illegitimate or synonymous in Jepson Manual (Ness 2011, in press)
~ unidentified taxon
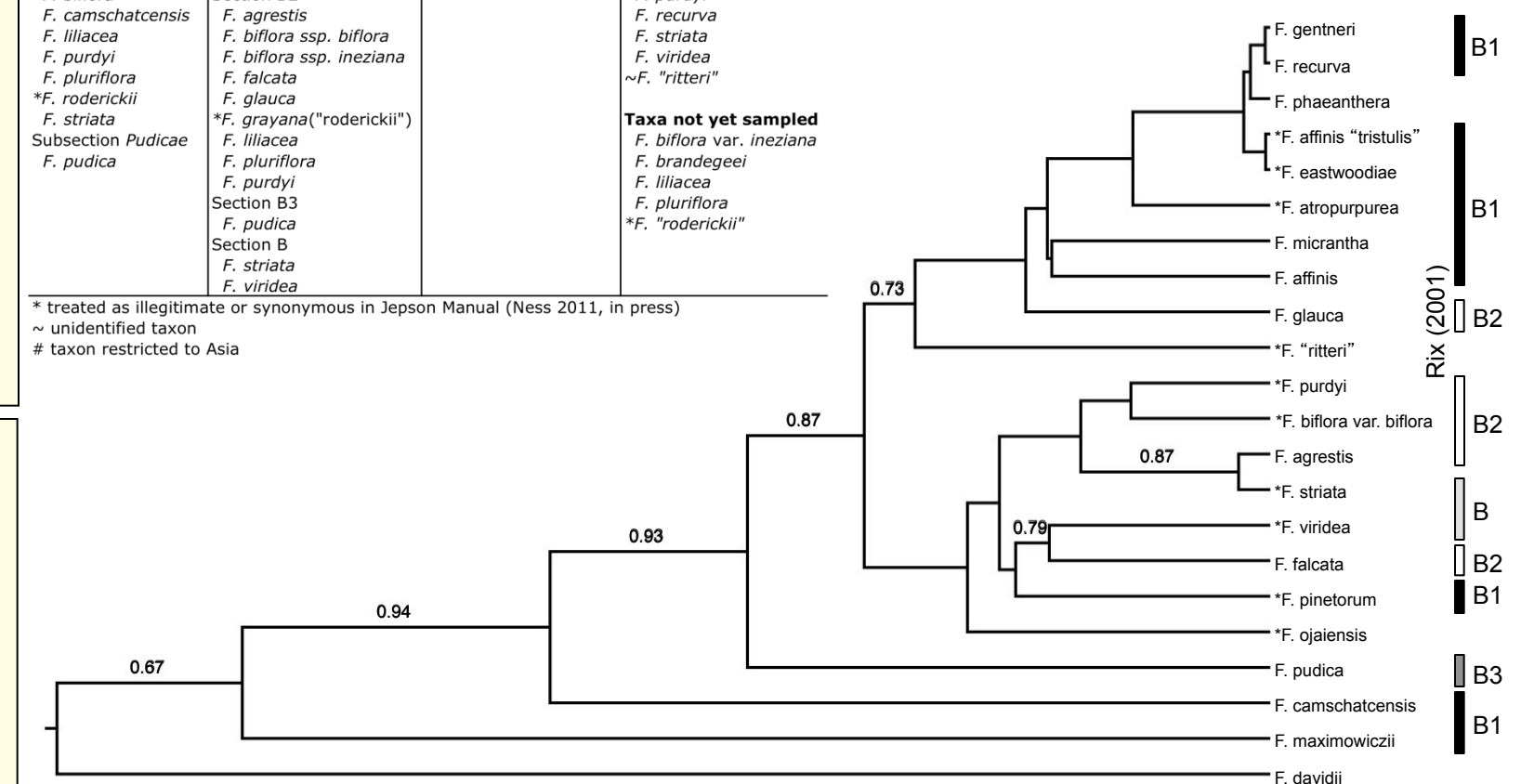\# taxon restricted to Asia

Figure 1. Maximum1clade credibility tree using *BEAST species tree estimation using ITS, matK, and rpl16 sequences for all currently available taxa. Only nodes with posterior probabilities >0.50 are shown. Note: taxa with an asterisk (*) lack a matK sequence.
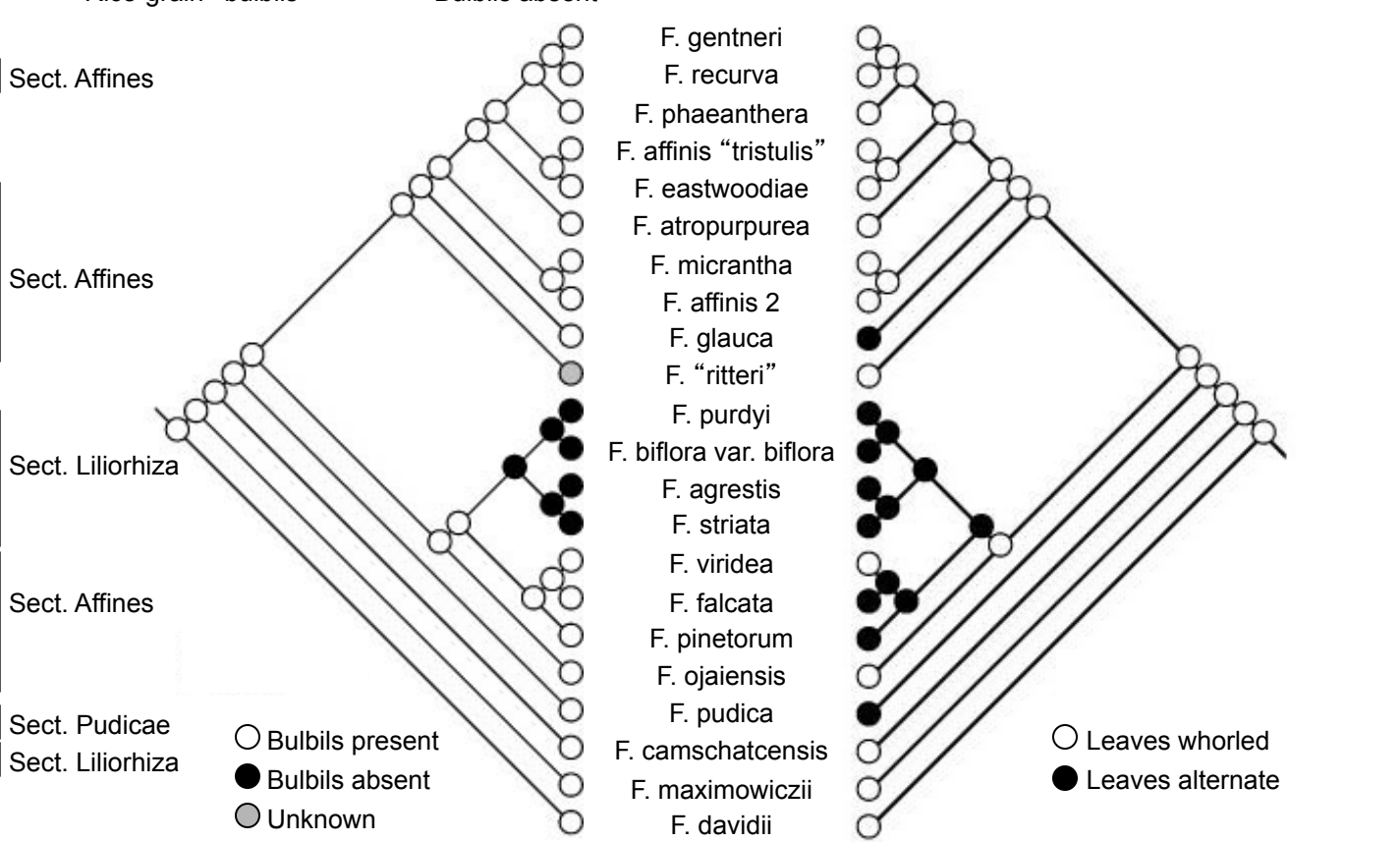
Figure 2. Parsimony ancestral character state reconstruction of "rice-grain" bulbils (left) and leaf arrangement (right).

Bulbils present
Bulbils absent
Unknown

Leaves whorled
Leaves alternate

## Literature Cited

Baldwin, B.G., S. Markos, 1998. Phylogenetic Utility of the External Transcribed Spacer (ETS) of 18S–26S rDNA: Congruence of ETS and ITS Trees of *Calycadenia* (Compositae). Molecular Phylogenetics and Evolution 10: 449-463.

Drummond, A.J. & A. Rambaut, 2007 BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7, 214

Drummond A.J., S.Y.W. Ho, M.J. Phillips, A. Rambaut, 2006. Relaxed Phylogenetics and Dating with Confidence. PLoS Biol 4(5): e88.

Maddison, W.P., D.R. Maddison, 2009. Mesquite: a modular system for evolutionary analysis. Version 2.72, available from http://mesquiteproject.org

Posada D., 2008. jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution 25: 1253-1256.

Rix, E.M., 2001. *Fritillaria*. A revised classification. The *Fritillary* Group of the Alpine Garden Society, United Kingdom.

Ronsted, N., S. Law, H. Thornton, M.F. Fay, M.W. Chase, 2005. Molecular phylogenetic evidence for the monophyly of *Fritillaria* and *Lilium* (Liliaceae; Liliales) and the infrageneric classification of *Fritillaria*. Molecular Phylogenetics and Evolution 35: 509-527.

Santana, D. O., 1984. Morphological and anatomical observations on the bulbs and tepals of *Fritillaria* (Liliaceae) Section *Liliorhiza* (Kellogg) Watson, their taxonomic implications with a synopsis and the reproductive biology of the section. Ph.D. dissertation. University of California, Davis.