

Elucidating evolutionary relationships in *Fritillaria* (Liliaceae)

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Introduction

- The c.140 species of *Fritillaria* are autumn germinating/spring flowering bulbous plants (Figs 1 & 2 show examples); the genus encompasses species of both medicinal and horticultural importance. Members of *Fritillaria* are found in the Northern Hemisphere's temperate zones of North America, Eurasia and North Africa.
- The most recent classification¹ recognised eight subgenera, with further sections and series within these. This classification was broadly supported by the results of a previous phylogenetic study² based on plastid and nuclear ribosomal region DNA sequence data, which recovered two major clades – one containing Eurasian, North American and North African species (seven subgenera) and the other subgenus containing mainly North American species.
- The discovery of giant genomes in several *Fritillaria* species^{3,4} has stimulated recent research on acquiring better knowledge of species relationships. Through recovery of increasingly resolved and well supported molecular phylogenetic trees for *Fritillaria*, a greater understanding of the relationships between species and subgenera is being achieved. This is an essential step in providing an evolutionary framework within which to study the mechanisms and evolutionary forces involved in genome size change, as well as forming an important part of wider studies of the genus.
- Sequence data for 37 *Fritillaria* species (c.26% of the genus) were included in the previously published phylogenetic work². In the current study, to date, DNA sequence data has been obtained for c.160 individuals, representing c.100 species (c.71% of the genus), as well as for 15 sister group (*Lilium*) and two outgroup species. Phylogenetic analysis, using maximum parsimony, has been performed.
- The latest results of this molecular phylogenetic work aimed at elucidating relationships between all species of *Fritillaria* are presented. Implications of these findings for our understanding of species relationships within the genus are discussed.

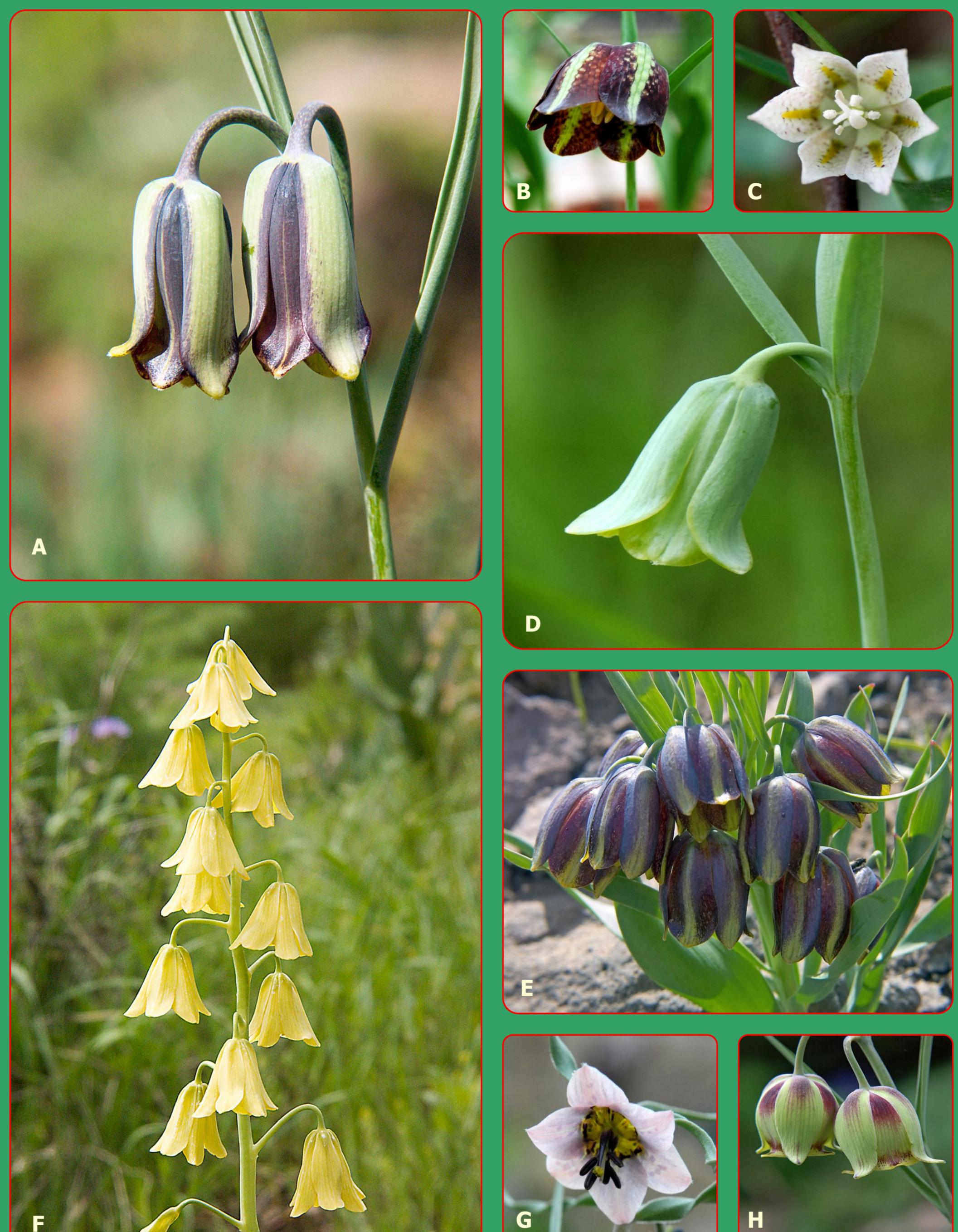


Figure 2. Examples of *Fritillaria* species sequenced: A) *F. latikensis* (subgen. *Fritillaria*); B) *F. thessala* (subgen. *Fritillaria*); C) *F. kaenensis* (subgen. *Japonica*); D) *F. alfredae* (subgen. *Fritillaria*); E) *F. crassifolia* (subgen. *Fritillaria*); F) *F. persica*, (subgen. *Theresia*); G) *F. gibbosa* (subgen. *Rhinopetalum*); H) *F. acmopetala* (subgen. *Fritillaria*). Photos A, E and H - R. Charman; B, C, D and G - L. Hill.

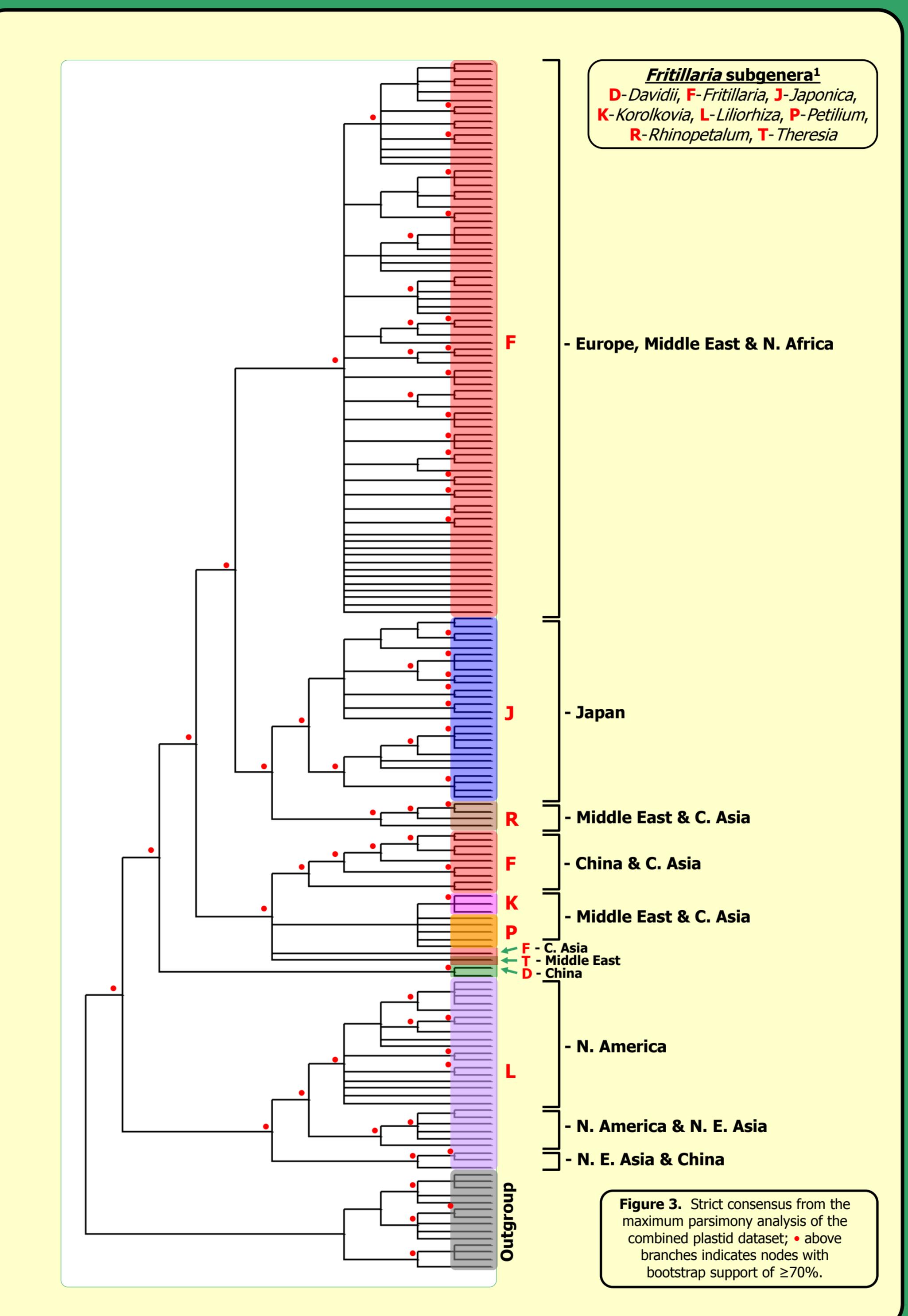


Figure 3. Strict consensus from the maximum parsimony analysis of the combined plastid dataset; ● above branches indicates nodes with bootstrap support of $\geq 70\%$.



Figure 1.

F. imperialis (subgen. *Petilum*)

R. Charman

Methods

- Three regions from the plastid genome (the *rpl16* intron and portions of the *rbcL* and *matK* genes) were amplified and sequenced from all available species and subspecies of *Fritillaria* (some examples sequenced are shown in Figs 1 & 2), including multiple individuals where possible. Representatives of *Lilium*, sister to *Fritillaria*, were also sampled.
- In addition to the plastid regions, the utility of the nuclear ribosomal ITS region was assessed; with results which showed that for *Fritillaria*, there is evidence for multiple sequence variants of ITS in many individuals, which limits the utility of this region for phylogenetics. For this study, inference of species relationships was therefore based only on the plastid sequence data.
- Phylogenetic trees inferred from individual plastid regions were compared for congruence before combining the data into a single plastid dataset. Maximum parsimony analysis was carried out using PAUP* v4.0 b10⁵.



Figure 4.

F. meleagris (subgen. *Fritillaria*)

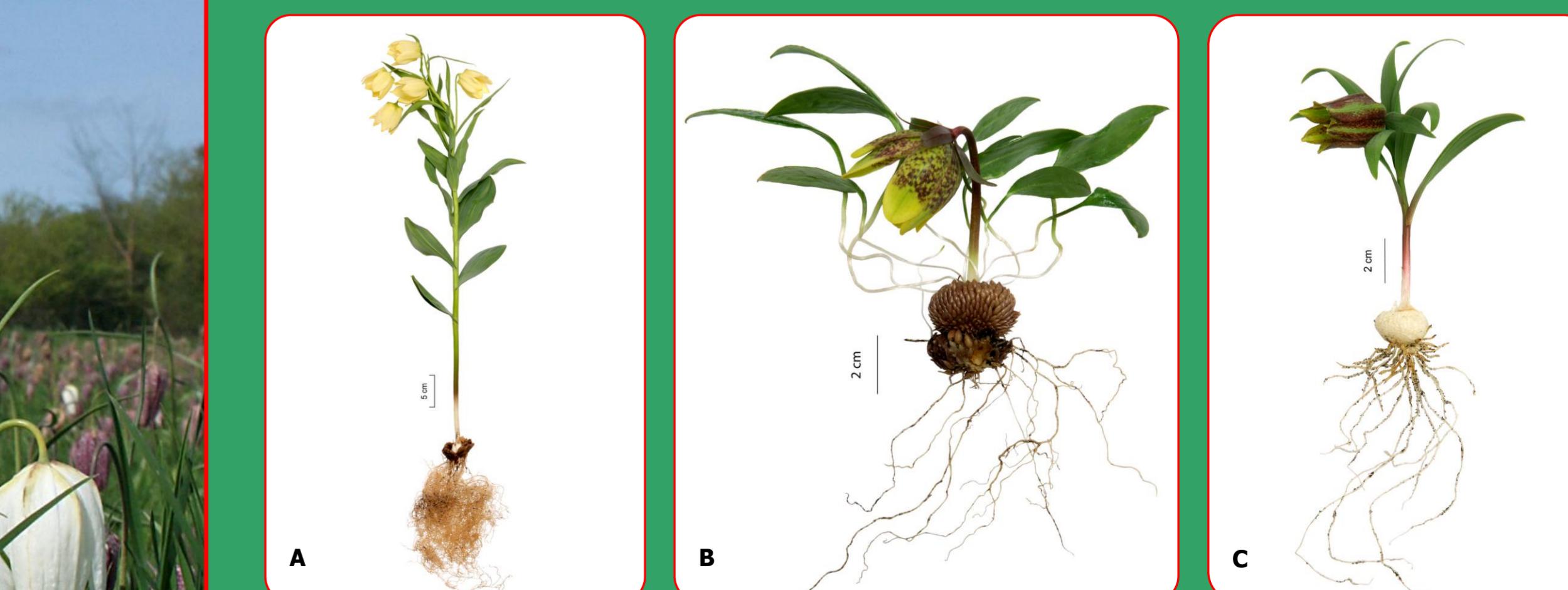


Figure 5. Examples of the range of morphological characters and states to be found within *Fritillaria*: A) *F. pallidiflora* (subgen. *Fritillaria*); B) *F. davidi* (subgen. *Davidii*); C) *F. kotschyana* (subgen. *Fritillaria*). Photos by L. Hill.

References

- (1) - Rix, E. M. (2001) *Fritillaria*: A revised classification. Alpine Garden Society, UK. (2) - Reinsted, N. et al. (2005) *Mol. Phylogenet. Evol.* **35**: 509-527. (3) - Leitch, I. J. et al. (2007) *J. Evol. Biol.* **20**: 2296-2308. (4) Ambrožová, K. et al. (2011) *Ann. Bot.* **107**: 255-268. (5) Swofford, D. L. (2003) PAUP*4.0b10.

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