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Fritillaria tubaeformis species complex (Liliaceae)

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Short title: A taxonomic revision of *Fritillaria tubaeformis*

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Abstract A morphometric multivariate and univariate study involving all the three taxa within the *Fritillaria tubaeformis* species complex was carried out. A total of 86 individuals from 8 populations were studied in vivo, complemented by the analysis of 116 individuals from herbarium specimens. According to our results, some morphological characters clearly support the separation among *F. burnatii*, *F. tubaeformis* and *F. moggridgei*. Despite this, some morphological overlapping does exist among *F. tubaeformis* and *F. moggridgei*, which show contiguous, partially interdigitated, but not overlapping ranges, and we deem more opportune their separation at subspecies level. On the contrary, *Fritillaria burnatii* is a clearly distinct species, albeit it can occasionally co-occur in the same site with *F. tubaeformis* subsp. *moggridgei*. An identification key for both fresh and dry specimens is provided.

Keywords endemics; Hautes Alps; herbarium specimens; in vivo specimens; Maritime Alps; morphological analysis; species delimitation; subspecies

Introduction

The genus *Fritillaria* L., with about 130 species, is the largest within Liliaceae tribe Lilieae Lam. & DC. (Peruzzi et al. 2009a; Peruzzi 2016). The latter tribe is composed by bulbous, herbaceous plants, with a bulb composed by 2-3 to many scales, anthers dorsifixed, fruit a loculicide capsule with seeds usually winged (Tamura 1998).

The tribe Lilieae is sister to Tulipeae (Chase et al. 1995; Patterson and Givnish 2002, Fay et al. 2006; Kim et al. 2013; Petersen et al. 2013), and the phylogenetic relationships among the large genera *Fritillaria* and *Lilium* were investigated by Rønsted et al. (2005). Studies more focused on *Fritillaria* were published by Türktaş et al. (2012), Day et al. (2014) and Kelly et al. (2015).

The genus, according to the classification proposed by Rix (2001), based on morphological characters, is subdivided into eight subgenera: *Fritillaria*, [including two sections: *Olostylae* Boiss. (six series) and *Fritillaria* (ten series)], *Rhinopetalum* Fisch., *Japonica* Rix, *Theresia* K.Koch, *Petilium* (L.) Endl., *Liliorhiza* (Kellogg) Benth. & Hook.f. (three series), *Davidii* Rix and *Korolkovia* Rix. This classification is supported by recent phylogenetic analyses (see above).

Fritillaria tubaeformis species complex is endemic of the Alps and belongs to *F.* subg. *Fritillaria* sect. *Fritillaria*. This complex has been variously treated by authors: a single variable species with no − or at most varietal − infraspecific taxa (Fiori 1923; Pignatti 1982; Aeschimann et al. 2004), a single species with two infraspecific taxa, *F. tubaeformis* Gren. & Godr. subsp. *tubaeformis* and *F. tubaeformis* subsp. *moggridgei* (Boiss. & Reut. ex Planch.) Rix (Zangheri 1976; Conti et al. 2005), three distinct taxa belonging to two different species: *F. tubaeformis* subsp. *tubaeformis*, *F. tubaeformis* subsp. *moggridgei*, *F. meleagris* L. subsp. *burnatii* (Planch.) Rix for Rix (1978, 1980),

F. tubaeformis subsp. *tubaeformis*, *F. tubaeformis* subsp. *moggridgei* and *F. burnatii* (Planch.) Backh. for (Noble and Diadema 2011; Tison et al. 2014). Finally, the taxa are treated as three different species by Tison and de Foucault (2014): *F. tubaeformis*, *F. moggridgei* (Planch.) Cusin, and *F. burnatii*. In accordance with previous literature on this species complex, Tison et al. (2014) and Tison and de Foucault (2014) have differentiated the three taxa on the base of the profile of the perigone, which is sub-rectangular in *F. tubiformis* s.l. and rounded in *F. burnatii*. Differences in tessellation intensity and colour of the tepals and in the average width of the leaf among the three taxa were also recorded.

The recent typification of the names involved (Bartolucci and Peruzzi 2012) clarified the identity of the three taxa with the following basionyms: Fritillaria tubaeformis Gren. & Godr., described from Hautes Alpes, France, F. delphinensis f. moggridgei Boiss. & Reut. ex Planch. and F. delphinensis var. burnatii Planch., both described from different localities in Maritime Alps. Recent molecular studies highlighted a clear distinctiveness concerning cpDNA *mat*K and *rpl*16 intron markers among the three taxa (Mucciarelli and Fay 2013), and also excluded any close relationship of *F. burnatii* with *F. meleagris* L., the latter species falling in a clade separate from the three taxa of the *F. tubaeformis* species complex, as more recently supported also by Day et al. (2014). Despite this, a RAPD population analysis revealed some genetic admixture among a few populations of *F. tubaeformis* subsp. *moggridgei* and *F. burnatii* (Mucciarelli et al. 2014).

This work is placed within a framework of taxonomic, systematic and conservation studies in the genus *Fritillaria*, carried out in recent years by our research group (Peruzzi et al. 2008, 2009b, 2012; Bartolucci et al. 2009; Peruzzi and Bartolucci 2009; Mancuso and Peruzzi 2010; Carasso et al. 2011, 2012, 2014; Bartolucci and Peruzzi 2012; Mancuso et al. 2012; Mucciarelli and Fay 2013; Mucciarelli et al. 2014). In order to clarify the taxonomic relationships among the three taxa of the *Fritillaria tubaeformis* species complex, we wanted to: (a) analyze the level of morphological differentiation within the *F. tubaeformis* group; (b) indicate the most informative characters for the identification of the three taxa; (c) compare fresh and herbarium specimens on the base of selected characters; (d) provide a key for fresh and dry specimens.

Materials and methods

Fresh plant material

Representatives of the three studied taxa were collected throughout their distribution areas (Fig. 1) in Maritime Alps (Italy) and in Hautes Alps (France). Twenty eight morphometric characters (24 continuous and 4 cardinal characters) (Table 1) were measured in the field from 86 flowering individuals (in vivo specimens). Fresh samples were collected from five populations of *F. delphinensis* var. *burnatii*, two populations of *Fritillaria delphinensis* f. *moggridgei* Planch. and one population from *F. tubaeformis* s.str. For sake of clarity and conciseness, the three taxa will be hereafter named as *F. burnatii*, *F. moggridgei* and *F. tubaeformis*, respectively. Only one-two herbarium vouchers from each population were collected, while most of the individuals were scored directly in field in order to allow for the least impact of sampling on population demography. Attention was paid to measure only flowers that had reached complete bloom and mature and fully expanded leaves. Samples from *loci classici* (known type localities) of the traditionally recognized taxa were Cima di Forte Pernante (Colle di

Tenda, Cuneo, Italy; acronym TEN), Valle Pesio (Chiusa di Pesio, Cuneo, Italy; acronym MAR) and col de Gleizé (Gap, France; acronym GLE) for *F. burnatii*, *F. moggridgei* and *Fritillaria tubaeformis*, respectively (Appendix 1). Morphological structures were measured with the use of an electronic digital calliper (0.01 digit; Millomex Ltd., UK).

Herbarium material

Additionally, eleven morphometric characters (7 continuous and 4 cardinal characters) (Table 1) were measured in 116 herbarium specimens for a total 202 individuals and 3702 measurements. The analysed samples were from the FI, G, G-BU, K, LY, P, PI and RO herbaria (acronyms according to Thiers, 2016). Type specimens were also analysed and added to the statistical analysis (Appendix 2). The number of characters scored on herbarium specimens was reduced with respect to those of fresh specimens because inner flower organs were accessible only in vivo. To minimize deterioration of this type of material, we measured a single outer and inner tepal to each herbarium specimen.

Statistical treatment of data

To assess that data conformed to requirements of normality and equality of variance the morphometric variables were tested for deviations from a normal distribution using a Kolmogorov–Smirnov test (p>0.05) (Cortinhas et al. 2015) in Origin Pro8 (OriginLab, Northampton, MA). Homogeneity of variance was assessed by a Levene's test. Quantitative continuous and quantitative discrete cardinal characters which did not meet the assumption for normality were log-transformed and square-root transformed, respectively, prior to be further analysed.

To reveal significant differences between the three taxa, a one-way analysis of variance (ANOVA) was performed in Origin Pro8 on two distinct data sets, one consisting of twenty-eight characters scored in field (in vivo data) and one of eleven characters scored on the herbarium specimens (herbarium data). A Tukey test was run for pairwise multiple mean comparisons of the characters. In the interspecific study, nine statistically variable characters common to the two data sets were combined (combined dataset) and used to detect differences between herbarium and in vivo specimens (SPECIMEN) and among the three taxa (TAXA) by means of a two-way ANOVA at the significant level of 0.05.

Descriptive and univariate analyses of morphometric variation

Simple descriptive statistics of infra-specific phenetic diversity (mean, standard deviation, standard error) were calculated for all variables. Box plots of median, mean, 25-75 percentiles, maximum and minimum values were computed with Orgin Pro8 on the most significant different characters of the two data sets for the sake of comparison between the studied taxa. For each variable measured in vivo, maximum and minimum values were calculated for each tepal type [MinOTL, MaxOTL, MinITL, MaxITL (outer and inner tepal lengths) and (MinOTW, MaxOTW, MinITW, MaxITW (outer and inner tepal widths)].

A further data set consisting of twenty-seven variant characters was prepared combining selected in vivo and herbarium characters and Pearson correlation coefficients (parametric) computed among all their pairs in order to check if any strong correlation ($r \geq 0.80$; p<0.01) existed that could potentially affect the results of further analyses (Dobeš et al. 2013; Ronikier and Zalewska-Gałosz 2014). For not normally

distributed variables, a Spearman correlation non-parametric method was applied (Šingliarová et al. 2011). To avoid redundancy in the data set, variables showing high correlation were removed resulting in a total matrix of sixteen variables for the multivariate analysis (multivariate data set).

Multivariate analyses

A principal component analysis (PCA) was conducted in Past 3.10 (Hammer et al., 2001) based on a variance-covariance matrix computed on the multivariate data set consisting of sixteen characters (all characters were log-transformed in order to compensate for variables having different measure units). Missing values originating from the differences in the number of observations between the two types of specimens were substituted by iterative imputation (Ilin and Raiko 2010). The analysis investigates the overall variation pattern along the first two components in order to find hypothetical variables (components) that can discriminate among groups. The axes extracted were those corresponding to components with eigenvalues greater than 1, which means that only components presenting a variation of at least one of the original variables are retained. The PCA results were presented as a two-dimensional scatter plot where each point represents one specimen.

In the next step, a classification discriminant analysis (CDA, cross-validation) was computed in *XL*Stat on the same data matrix. The analysis was performed in order to determine the highest probability membership group of the samples (Legendre and Legendre 1998). Three groups were a priori defined according to their taxonomic affiliation. Group assignment was cross-validated by a leave-one-out cross-validation (Jackknifing) procedure. Both, the identification of the more discriminating variables by means of Fisher's coefficient ($p<0.05$), the posterior probability of classification of each sample and the Wilks' Lambda value were calculated as a measure of the discriminant power in *XL*Stat. A Wilks' Lambda value close to zero indicated a better discrimination between the predefined groups (Torrecilla et al. 2013).

Results

Morphometric variation within *F. tubaeformis* complex.

In this study a total of thirty nine morpho-anatomical characters chosen based on selected phenotypic characters known to be representative of the studied taxa were analyzed in 202 specimens of *Fritillaria tubaeformis* species complex. Measurements were conducted directly in field on the most typical populations of the three taxonomic groups, and a large dataset was obtained also analyzing herbarium specimens in consideration of the valuable information this type of data provide and of its relevance for both conservation and classification purposes.

Morphometric variation of in vivo specimens.

Results of descriptive statistics and ANOVA showed that thirteen of the characters measured in vivo significantly differentiated the three studied taxa according to their phenetic diversity showing *F* values greater than 25.00 ($p\leq0.001$) (Table 2; Fig. 2). With regard to the perigone, maximum and minimum average lengths and widths of both outer and inner tepals (MinOTL, MaxOTL, MinOTW, MaxOTW, MinITL, MaxITL, MinITW and MaxITW) together with maximum and minimum average lengths of their nectaries (MinONL, MaxONL, MinINL and MaxINL) were all highly

significantly different (Table 2). Lengths of tepals ranged within 35.61−37.51 mm and 35.13−37.09 mm in *F. burnatii* (outer and inner tepals, respectively) and, according to the Tukey HSD test, were significantly shorter (F>25.00, p=0.000) than in *F. tubaeformis* (43.10−44.82 and 42.94−44.98 mm outer and inner, respectively) and in *F. moggridgei* (41.11−43.02 and 43.07−44.78 mm, respectively) (Figs. 2a, b, 3a−c; Table 2). Values for the width of the inner tepals (MinITW and MaxITW) also differed significantly accounting for 13.76−15.43 mm in *F. burnatii*, significantly lower (F>25.00, p=0.000) than in *F. tubaeformis* (21.63−24.36 mm) and in *F. moggridgei* (22.86−24.82 mm) (Figs. 2c, 3a−c; Table 2). According to the average width of the outer tepals (MinOTW and MaxOTW) and to the length of the corresponding nectaries (MinONL and MaxONL), the three taxa differed significantly among them all (F>15.00, p=0.000; Figs. 2d, e, 3a−c; Table 2). On the contrary, *F. moggridgei* and *F. burnatii* differed significantly from *Fritillaria tubaeformis* according to the length of innermost nectaries (MinINL and MaxINL; F>15.00, p=0.000; Fig. 2f; Table 2).

The width of the largest leaf (MaxLW) measured on average 7.99±0.22 mm in *F. burnatii* and resulted significantly different (F=76.701, p=0.000) from the average largest leaf of *F. tubaeformis* (13.94±2.44 mm) and *F. moggridgei* (16.43±1.31 mm) (Figs. 3a−c, 4; Table 2).

Ovaries, styles, stigmas, stamen filaments and anthers always showed average lengths statistically different among taxa, although with F values lower than in vegetative characters (F=8.813−24.662, p=0.000; Table 2). However, according to these characters, the three taxa differed variously among them (see over in results).

Morphometric variation in herbarium specimens

When the ANOVA was run on the large herbarium data set, the number of basal alternate, bracteal and total leaves (LalterN_herb, LbractN_herb and LN_herb) and the width of outer nectaries (ONW_herb) were not normally distributed and therefore were transformed prior to analysis of variance. Five of the eleven characters analyzed [outer tepal length (OTL_herb), outer tepal width (OTW_herb), inner tepal length (ITL_herb), inner tepal width (ITW_herb), width of the largest basal leaf (MaxLW_herb)] showed highly significant variation among taxa (F≥19.00, p=0.000) (Table 3). Tepals (OTL_herb and ITL_herb, outer and inner, respectively) were always shorter and the largest leaf (MaxLW_herb) narrower in *F. burnatii* (33.86±0.47, 33.75±0.51 and 4.47±0.22 mm, respectively) than in *F. moggridgei* (38.08±0.67, 38.87±0.70 and 9.12±0.52 mm, respectively) and *F. tubaeformis* (39.78±1.09, 39.93±1.04 and 9.05±0.55 mm, respectively) (p=0.000) (Table 3). According to the outer tepal width (OTW_herb), *F. burnatii* and *F. moggridgei* were not statistically different and according to the inner tepal width (ITW_herb) all the three taxa differed significantly (F=63.11, p=0.000; Table 3). Color and tessellation of the perigone were excluded from the comparison *ab initio* because these characters were difficult to be judged, especially in dried specimens.

Comparison between in vivo and herbarium specimens

Eight characters of the combined dataset showed significant differences when measured in in vivo vs. herbarium specimens (SPECIMEN, p=0.000) (Fig. 5; Table 4). Specifically, mean values of tepal lengths (39.20±0.31 and 39.39±0.35 mm, OTL and ITL, respectively), tepal widths (14.48±0.19 and 18.34±0.33 mm, OTW and ITW, respectively), the number of basal and total leaves (4.86±0.09 and 5.87±0.08, LalterN

and LN, respectively) and the width of the largest leaf (11.03±0.53 mm, MaxLW) were significantly larger when measured in in vivo than in herbarium specimens (36.78±0.46 mm, 37.05±0.4 mm, 11.23±0.24 mm, 15.02±0.34 mm, 4.35±0.08 and 5.83±0.08 mm, respectively) (Fig. 5; Table 4). However, the two-way ANOVA showed that these characters differentiated the three taxa (TAXA, F= 4.829−241.546, p≤0.01) also when compared based on the combined dataset. Differences in lengths of the flower pedicel did not receive statistical support (Table 4).

With regard to the leaves, in this study we found that the average number of basal alternate leaves ranged within 4.84−5.27 per plant with little statistical support with regard to differences among the three taxa (F = 3.424, p = 0.037; Table 2). The number of bracteal leaves per plant was invariably equal to one, thus the total number of leaves ranged between 5 and 6 (Table 1). In herbarium specimens, the number of basal alternate and total leaves ranged within 4.15−4.64 and 5.15−5.68 per plant, respectively, with very little statistical support with regard to the differences between taxa (F=3.738, p=0.027 and F=3.154, p=0.046, respectively) (Table 3). Unexpectedly, herbarium specimens had on average fewer leaves than the in vivo specimens did (SPECIMEN, p=0.000) (Table 3) probably due to accidental losses during transport of dried samples.

On the contrary, the average plant height in specimens from herbaria largely exceeded that of in vivo specimens (193.27±4.27 and 160.30±3.45 mm, respectively; SPECIMEN, p=0.000), while differences among taxa did not receive statistical support in the combined dataset (TAXA, p=0.795) (Table 3).

Based on ANOVAs results, eight variables from the matrix on in vivo specimens, namely plant height, number of flowers, pedicel length, number of bracteal leaves, maximum and minimum width of nectaries of both external and internal tepals, and four variables from herbarium specimens, namely plant height, number of flowers, number of bracteal leaves and flower pedicel length were excluded from the following analysis because not statistically different between the three taxa (p≥0.05) (Table 2−4). To avoid redundancy in the data set, of the eight variables on tepals showing high correlation coefficients (*r*=0.92−0.95, p≤0.000), six characters were removed from the analysis, maintaining the maximum length and width of inner tepals measured in vivo (MaxITL and MaxITW) and the length of inner tepals (ITL_herb), and the width of both outer and inner tepals for the herbarium specimens (OTW_herb and ITW_herb). Variables concerning the total (LN) and basal alternate leaf (LalterN) numbers were highly correlated (*r*>0.99; p≤0.001 both of in vivo and in herbarium specimens), thus, only one of each pair was retained (LN and LN_herb, respectively). Maximum and minimum nectary lengths of internal and external tepals were highly correlated (*r*>0.94 and *r*>0.91, p≤0.001, internal and external, respectively), therefore only maximum lengths of nectaries of inner (MaxINL) and outer tepals (MaxONL) were considered. Thus in the end, eleven characters were removed resulting in a total matrix of sixteen variables for the multivariate analysis (multivariate data set).

Results of the multivariate analyses

In the PCA analysis two axes were designated accounting altogether for 100% of the variance, whose scatterplot is shown in Fig. 6. The first component explained 69.28% of the total variation while the second component explained 30.72%. Samples segregated into three main clusters corresponding to *F. burnatii*, *F. tubaeformis* and *F. moggridgei*, however a substantial overlapping was present between these last two taxa in the right part of the PCA plot (Fig. 6).

The original characters explaining most of the variation in the first component (PC1) belonged to both type of data matrices. For herbarium specimens they were inner tepal length (ITL_herb, component loading 0.298), inner tepal width (ITW_herb, 0.298) and width of the largest leaf (MaxLW_herb, 0.280). Similarly, the variables measured *in vivo* giving a major contribution to PC1 were maximum inner tepal length (MaxITL, 0.264), maximum inner tepal width (MaxITW, 0.258), width of the largest basal leaf (MaxLW, 0.243) and with the addition of style length (0.263). According to these results, *F. burnatii* lied completely in the left part with negative values of the PCA plot, well separated from *F. tubiformis* and to a lesser extent from *F. moggridgei* (Fig. 6).

Characters measured on *in vivo* specimens, instead, were the principal contributors to Component 2 of the PCA analysis. The latter, in fact, was mainly influenced by anther (component loading 0.444), ovary (0.370) and filament length (0.274) which mainly contributed to the separation between *F. moggridgei* and *F. tubaeformis*.

The results of the Discriminant Analysis are shown in Fig. 7. Three clusters are recognizable on the CDA plot; *F. burnatii* points showed no overlap with *F. moggridgei* data, while a moderate data overlapping was present between *F. moggridgei* and *F. tubaeformis* in the right part of the plot (Fig. 7a). Separation of *F. burnatii* from both *F. moggridgei* and *F. tubaeformis* on the discriminant Function 1, which accounted for 72.79 % of the total variation, was supported by at least five characters measured on in vivo specimens. These characters were the maximum width and length of inner tepals (eigenvalues 0.848 and 0.751, respectively), the width of the largest leaf (eigenvalue 0.782) and the length of the ovary and style (eigenvalues 0.420 and 0.385, respectively) (Figs. 6a, b). This separation along the CDA Function 1 was supported also by the same three characters when measured in herbarium specimens (eigenvalues 0.789 for the width of the largest leaf; 0.782 and 0.571 for the width and length of inner tepals, respectively) and by the width of the outer tepals (eigenvalue 0.542) (Figs. 7a, b). *Fritillaria moggridgei* and *F. tubaeformis* samples separated, although not totally, with respect to the CDA Function 2 which accounted for 27.22 % of the variation. All the most discriminating characters on the CDA Function 2 were measured on in vivo specimens. These corresponded to anther and ovary lengths (eigenvalues 0.592 and 0.361, respectively) in the case of *F. moggridgei* and *F. tubaeformis*, and to the maximum lengths of nectaries (eigenvalues 0.552 and 0.474, outer and inner tepals, respectively), together with stamen filament and stigma (eigenvalues 0.434 and 0.413, respectively) in the case of *F. burnatii* samples. The low Wilks' lambda value (0.023, p≤0.001) supported the clear phenetic separation between *F. burnatii* and the other two taxa.

The discriminant analysis classification method based on the analysed characters resulted in the correct classification of 99.05 % (86.97 % after cross validation) of the whole samples. Samples of *F. burnatii* were correctly classified into their respective group in the 100 % of cases (still 100 % after cross validation), while samples of *F. moggridgei* in the 96.3 % (70.37 % after cross validation) and *F. tubaeformis* in the 100 % of cases (78.57 % after cross validation) might result reciprocally correctly classified.

Discussion

Morphological differentiation

The morphometric study of *F. tubaeformis* species complex confirmed the differentiation of the three taxa – *F. burnatii*, *F. moggridgei* and *F. tubaeformis.* Tepals,

pistils and stamens, other than leaf size showed variation among individuals assigned a priori to one of the taxonomic groups, pointing to consistent and statistically supported differences among them.

As a main finding in this regard, outer and inner tepals in *F. burnatii* were consistently shorter (inner tepals also narrower) than in *F. moggridgei* and *F. tubaeformis*. Instead, according to the width of outer tepals, *F. moggridgei* showed to be intermediate between the other two taxa (Fig. 3). These results supported flower descriptions found in Italian and French floras for the *F. tubaeformis* species complex (Pignatti 1982; Tison and de Foucault 2014; Tison et al. 2014). In fact, perigone having a typical U profile with tepals pointed to the apex and shorter than in *F. moggridgei* and *F. tubaeformis*, are the main features of *F. burnatii*. A perigone with a sub-rectangular profile and angled nectaries being, instead, characteristic of *F. moggridgei* and *F. tubaeformis*. These observations suggest the idea that in these taxa, nectary length and shape could be related to the tepal length. Our data did not confirm this occurrence because nectary lengths were inversely correlated to both tepal lengths and widths ($r=-0.352$ and $r=-0.459$, $p \leq 0.001$, respectively). In fact, *F. moggridgei* and *F. tubaeformis* showed longer tepals and shorter nectaries with respect to *F. burnatii* (outer and inner tepals) (Figs. 2 e, f; Table 1).

Although our measurements can only tentatively represent differences in perigone shape, this is the first time in which phenetic differences of these organs have been taken into consideration for taxa delimitation in *F. tubaeformis* species complex.

Taxonomic value of the combined datasets

Herbarium data in most cases provide reliable information for rare plant species assessment. Critical taxa descriptions and identifications can be coupled with temporal, spatial, and abundance data contained in most herbarium collections thus allowing identification of species deserving conservation attention and/or further study (Cortinhas et al. 2014; Kricsfalusys and Trevisan, 2014 and references therein).

Aiming at a redefinition of the taxonomical status of *F. tubaeformis* species complex, in this study, field observations were implemented with measurements on herbarium specimens. Results of in vivo analysis found strong statistical support when analysed in the herbarium counterpart. In fact, although significantly different between the two types of specimens, outer and inner tepals were consistently shorter and, inner tepals also narrower in *F. burnatii* than in *F. moggridgei* and *F. tubaeformis* (compare Tables 1 and 2). Results of the two-way analysis of variance supported these occurrences and showed that tepal size and leaf width differentiated the three taxa even when based on the combined dataset. Size reduction in herbarium specimens was almost probably a consequence of tissue shrinkage during air-drying of fresh specimens. Tepal and leaf size, nonetheless were dimensionally consistent within each species, wherever they were measured, and thus taxonomically meaningful. Only according to the width of the outer tepals, a discrepancy was found, and *F. moggridgei* did not differ from *F. burnatii* (herbarium specimens) and it was intermediate between the other two taxa (*in vivo* specimens) (Tables 1, 2).

Taxonomic position of *Fritillaria burnatii*

The PCA analysis agreed with the delimitation of three taxa within the *F. tubaeformis* species complex proposed by Tison et al. (2014) and Tison and de Foucault (2014) and the Discriminant Analysis confirmed the usefulness of the studied phenetic characters

when combined in a single matrix (Fig. 6, 7). Based on the organization of the perigone, *F. burnatii* positioning vs. *F. moggridgei* and *F. tubaeformis* on the function 1 of the CDA plot was determined by the width and length of inner tepals (both in vivo and herbarium specimens; Fig. 7b), and by the outer tepal width measured in herbarium specimens in the case of *F. tubaeformis*.

Variation in reproductive organs

With regard to the morphometry of the reproductive organs, the analysis of variance showed a variegated situation within the three taxa. Differently from tepals, ovary, style, stigma, stamen filament and anther lengths showed no apparent relation with the separation between *F. burnatii* from the other two taxa. In fact, based on the analysis of variance, *F. moggridgei* showed ovaries, stigmas, anthers and stamen filaments statistically longer than *F. tubaeformis*, but not the style (Table 2). *Fritillaria moggridgei*, on the other hand, seemed to share with *F. burnatii* the stigma and the stamen filament, which lengths did not differ statistically. Reproductive characters, however, demonstrated to be important determinants in the multivariate analysis where: 1) differences in anther and ovary lengths separated *F. moggridgei* from both *F. tubaeformis* and *F. burnatii*; 2) according to stigma and filament lengths, *F. moggridgei* was closer to *F. burnatii* (Fig. 7). Pistil and particularly the stigma morphology are phenotypic reproductive traits known to be representative of the inter- and intraspecific variation within Liliaceae (Peruzzi 2016), but have been poorly or at all employed so far within the genus *Fritillaria*. No data in literature are available on the situation in *F. tubaeformis* species complex. Tison and de Foucault (2014) did not cite any futures in this regard and, Pignatti (1982) generally referred to a style 12−13 mm and stigmas 2 mm long. According to our results, mean values for the style reported by Pignatti (1982) are closer to the situation found in *F. tubaeformis* than in *F. burnatii* (Table 2).

Leaf size and species differentiation

Leaf number, shape of the lamina and position on the stem are all characters having gained much attention in the past for taxa delimitation within *F. tubaeformis* species complex. Tison and de Foucault (2014) and Tison et al. (2014) have remarked that in *F. tubaeformis* s.str., cauline leaves aggregate in the upper part of the stem as typical of all these "Alpine" fritillaries. Besides, in *F. tubaeformis* the lowermost leaves are generally flat or just curled at the margins, often coiling at the apex and usually larger than 7 mm, differently from *F. burnatii* where leaf laminas are rather curled at margins, never coiling at the apex and usually less than 7 mm in width. This study has confirmed these differences, in fact we found that *F. burnatii* leaves were significantly narrower (7.99±0.22 and 4.47±0.22 mm, in vivo and in herbarium specimens, respectively) than in *F. tubaeformis* and *F. moggridgei* (Fig. 4; Table 2−3). Leaf width received also high statistical support by the multivariate analyses separating data into two groups, one consisting of *F. burnatii* samples located in the left part of negative values and one of *F. tubaeformis* and *F. moggridgei* samples, laying in the right part of the positive values of PCA and CDA plots.

General Conclusions

Results of this study have clearly indicated that the three taxa within *F. tubaeformis* species complex present many intermediate morphological characters. However, we

demonstrated that for some characters, a clear separation between *F. burnatii* and *F. moggridgei* + *F. tubaeformis* is feasible. Considering that either the two data matrices, employed in this study, showed their own limits and benefits, we have demonstrated that characters with high taxonomic value as such as tepal dimensions can be properly used for taxonomy in *Fritillaria* species.

   F. tubaeformis s.l. is increasingly rare in the wild due to habitat loss. The implementation of genetic data retrieved from in field samplings with DNA obtained from herbarium specimens would represent in future a valuable instrument to expand our knowledge on geographical and temporal patterns of diversity within these species.

Taxonomic Treatments

According to our study, there are some morphological characters that clearly support the separation among *F. burnatii*, *F. tubaeformis* and *F. moggridgei*, albeit with some overlapping, especially between the latter two taxa. Considering that *F. tubaeformis* and *F. moggridgei* are morphologically very close and show contiguous, partially interdigitated, but not overlapping ranges, we deem more opportune their separation at subspecies level, in agreement to the taxonomic treatment recently proposed by Tison et al. (2014). *Fritillaria burnatii* and *F. moggridgei*, on the contrary, can occasionally co-occur in the same site. This may account for a certain degree of genetic admixture found among some populations of *F. tubaeformis* subsp. *moggridgei* and *F. burnatii* (Mucciarelli et al. 2014).

Fritillaria burnatii (Planch.) Backh., Gard. Chron., n.s., 11: 685 (1879).
≡ *Fritillaria delphinensis* var. *burnatii* Planch., Bull. Soc. Bot. France 20: 115 (1873) ≡ *Fritillaria delphinensis* subsp. *burnatii* (Planch.) K.Richt., Pl. Eur. 1: 212 (1890) ≡ *Fritillaria tubaeformis* var. *burnatii* (Planch.) Rouy, Fl. France 12: 403 (1910) ≡ *Fritillaria meleagris* L. subsp. *burnatii* (Planch.) Rix, Bot. J. Linn. Soc. 76: 356 (1978).
- *Fritillaria meleagris* Ambros., Fl. Tirolo Mer. 1: 530 (1854), non L.
Lectotype (designated by Bartolucci & Peruzzi 2012: 28): FRANCE. Maritime Alps: Pâturages au mont Piernaude pres le col de Tende, 29 June 1861, *E. Bourgeau 346* (G-BU barcode G00096148!, complete individual on the right; isolectotypes: FI!, G barcodes G00169894!, G00169896!, G00169897!).
   Distribution: Endemic to southwestern and central Alps (Fig. 1): Maritime Alps and Cottian Alps (France, Italy), Pennine Alps (Italy) and Rhaetian Alps (Italy). Baker (1875, as *F. delphinensis* var. *burnatii*) recorded this species also for Corsica, based on a specimen collected by Dierek and kept in the Moggridge's herbarium at K. This citation was later reported by Richter (1890, as *F. delphinensis* subsp. *burnatii*), Arcangeli (1894, as *F. delphinensis*) and Fiori & Paoletti (1896, as *F. delphinensis*). Rouy (1910), in a note to *F. tubiformis* var. *burnatii*, writes: "*Le F. tubiformis a été indiqué en Corse: nous n'avons aucune donnée sur la présence de cette Fritellarie dans notre grande île méditerranéenne; et sa présence nous y parait peu vraisemblable*". After Rouy, indeed, no one has indicated any *Fritillaria* from Corsica (see also Jeanmonod and Gamisans, 2007; Tison and de Foucault 2014). We were not able to trace specimens from Corse at K (Odile Weber, *in litt.*). However we found one specimen at P (barcode P01776694!, available at https://science.mnhn.fr/institution/mnhn/collection/p/item/p01776694?listIndex=4&list Count=4). This specimen belongs indeed to *F. burnatii*, by admitting that the locality

appearing on the label (Corse – Bonifacio, June 1919, *Bicknell 1662*) is correct. However, we deem the label of this specimen as not reliable.

Fritillaria tubaeformis Gren. & Godr., Mém. Soc. Émul. Doubs, sér. 2, 6: 13 (May 1854, publ. 1855) subsp. ***tubaeformis***
≡ *Fritillaria delphinensis* Gren. in Grenier and Godron, Fl. France 3: 180 (ante June 1855), *nom. illeg.* Lectotype (designated by Bartolucci and Peruzzi, 2012: 24): FRANCE. Hautes Alpes: Lusette en Luz (Drôme), 1847, *Boullu s.n.* (P barcode P00654037!).
= *Fritillaria delphinensis* var. *reverchonii* Rouy, Fl. France 12: 403 (1910).

   Distribution: Endemic of the French southwestern Alps (Fig. 1): from Maritime Alps (west to the Tinée valley) and provencal Prealps near Grasse to Dauphiné Prealps near Gap. Grenier and Godron (1855), in the protologue cite *F. tubaeformis*, quote also from Mt. Viso (Cottian Alps). However, a specimen collected by Grenier in 1842 from Mt. Viso (P barcode P00654036!) is to be referred to *F. burnatii*. Hence, according to field investigations and the studied herbarium specimens we exclude the presence of *F. tubaeformis* from that area.

   Notes: Many authors quote the specific epithet as "*tubiformis*", but in accordance to the protologue and according to Art. 60.8 and Rec. 60G.1(c) of the ICN (McNeill et al. 2012), the correct spelling is "*tubaeformis*".

Fritillaria tubaeformis subsp. ***moggridgei*** (Planch.) Rix, Bot. J. Linn. Soc. 76: 356 (1978)
≡ *Fritillaria delphinensis* Gren. f. *moggridgei* Boiss. & Reut. ex Planch., Bull. Soc. Bot. France 20: 116 (1873) ≡ *Fritillaria moggridgei* (Boiss. & Reut. ex Planch.) Cusin, Herb. Fl. Fr. 21: t. 12 (1876) ≡ *Fritillaria delphinensis* var. *moggridgei* (Boiss. & Reut. ex Planch.) Nyman, Consp. Fl. Eur.: 722 (1882) ≡ *Fritillaria delphinensis* subsp. *moggridgei* (Boiss. & Reut. ex Planch.) K.Richt., Pl. Eur. 1: 212 (1890) ≡ *Fritillaria tubaeformis* var. *moggridgei* (Boiss. & Reut. ex Planch.) Rouy, Fl. France 12: 404 (1910).
Lectotype (designated by Bartolucci and Peruzzi 2012: 24): ITALY. Maritime Alps: Col de Carbon, près le val Pesio (Prov. de Cuneo), Piémont, 29/VII/1872, *E. Burnat s.n.* (G-BU barcode G00096153! individual on the left; isolectotype: G-BU barcode G00096152!).

   Distribution – Endemic of southwestern Alps (Fig. 1): Maritime Alps (France, Italy), Cottian Alps (France, Italy).

keys to *Fritillaria tubaeformis* species complex

For fresh specimens:

1a. Leaves (4.4)6.3-9.6(13.5) mm wide; perigone with U profile, tepals pointed at apex (acute or obtuse), purple with evident white tessellation; outer tepals (23.7)32.2-42.5(46.2) × (7.1)10.8-16.1(20) mm, inner tepals (21.3)31.1-41.6(41.6) mm × (5)11-18.1(18.1) mm, style (3.1)5.4-10.8(12.7) mm long... ***F. burnatii***
1b. Leaves (9)11-21.2(32) mm wide; perigone with sub-rectangular profile, tepals rounded at apex, yellow or purple lacking a white tessellation, outer tepals (33.5)37.8-47.1(50.9) × (10.8)13.3-19.9(22.9) mm, inner tepals (33.9)39.5-48.3(52.5) × (17.7)20.6-

27.4(30.7) mm, style (3.6)6.4-13.2(16.6) mm long...……...2 (*F. tubaeformis* s.l.)
2a. Tepals yellow, sometimes with purple tessellation; ovary (7.9)8.9-12.2(12.2) mm, stigma lobes (1.1)2.9-5.3(5.4) mm, anthers (5.9)8.6-14.6(15) mm long ………..………… ***F. tubaeformis*** subsp. ***moggridgei***
2b. Tepals uniformly purple, glaucous, tessellation mostly hidden by pruina; ovary (5.9)6.6-9.8(13.5) mm, stigma lobes (1.1)1.4-2.8(3.1) mm, anthers (5.5)5.9-8.2(9.2) mm long …………………………………………..... ***F. tubaeformis*** subsp. ***tubaeformis***

For dried specimens:

1a. Leaves (1.7)2.9-6.5(8.8) mm wide; outer tepals (27.2)30.1-38(43.6) × (8)9-11.8(13.3) mm, inner tepals (27)30-38(43.7) × (9)10-15.3(16) mm..***F. burnatii***
1b. Leaves (4.7)5.5-13.1(18.9) mm wide; outer tepals (30.1)32.6-46.0(49) × (7)9-16.4(22) mm, inner tepals (30)32.8-46(48) × (10.5)13-21.1(26) mm...2 (***F. tubaeformis*** s.l.)
2a. Inner tepals (10.5)13-18(24.2) mm wide, outer tepals (7)8.5-11.8(13.6) mm wide; perigone yellow ....…. ***F. tubaeformis*** subsp. ***moggridgei***
2b. Inner tepals (13)14.7-24.3(26) mm wide, outer tepals (9)10.2-18.8(22) mm wide; perigone purple …………....................................…. ***F. tubaeformis*** subsp. ***tubaeformis***

Appendix 1. List of the populations within *Fritillaria tubiformis* species complex investigated for the morphological analysis on *in vivo* specimens (country, locality, coordinates of provenance, date, scientists). Voucher specimens are kept in TO and PI. Acronyms and herbar vouchers are indicated in brackets for each population.

F. burnatii – **Italy** – Alpi Liguri – Alpe Pian Rosso (Cuneo), 44°09′ 007°46′, 1550–1650 m, 1 June 2013, *M. Mucciarelli* et *W. Camusso* (acronym ROS, TO-HP 7479); Alpi Cozie – San Michele di Prazzo (Cuneo), 44°31′ 007°02′, 2000–2020 m, 14 June 2013, *M. Mucciarelli* et *W. Camusso* (acronym PRA, TO-HP 7480); Alpi Marittime – Cima di Forte Pernante (Mt Piernaud) (Cuneo), 44°14′ 007°54′, 1898–2010 m, 16 June 2013, *M. Mucciarelli* et *W. Camusso* (acronym TEN, locus classicus, TO-HP 7481); Alpi Liguri – Vallone di Serpentera (Cuneo), 44°13′ 007°41′, 1870–1920 m, 21 June 2013, *M. Mucciarelli* et *W. Camusso* (acronym SER, TO-HP 7482); Alpi Cozie – Valle Varaita, dopo borgata Foresto (Cuneo), 44°60′ 007°15″, 1770–1780 m, 7 June 2014, *M. Mucciarelli* et *P. Rosso* (acronym FOR, TO-HP 7483).

F. moggridgei – **Italy** – Alpi Liguri – Pian del Lupo (Cuneo), 44°11′ 007°41′, 1990 m, 17 June 2014, *M. Mucciarelli* et *P. Rosso* (acronym PLU, TO-HP 7484); Alpi Liguri – Valle Pesio (Cuneo), 44°11′ 007°40′, 1470 m, 1 June 2014, *M. Mucciarelli* et *W. Camusso* (acronym MAR, locus classicus, TO-HP 7485).

F. tubaeformis s.str. – **France** – Hautes-Alpes – col de Gleizé (Gap), 44°37′ 006°02′, 1790 m, 16 May 2015, *M. Mucciarelli* et *P. Rosso* (acronym GLE, locus classicus, TO-HG 3328).

Appendix 2. List of herbarium specimens of *Fritillaria tubaeformis* species complex used in the morphological study (country, locality, date, collectors, herbarium code, barcode when available).

F. burnatii – **France** – Alpes Maritimes – Authion, 22 June 1860 Fr. le 13 July 1863, *Canut s.n.* (FI!); Alpes Maritimes, paturages pres le col de Tende, June 1874, *Beltrandi s.n.* (FI!); Alpes Maritimes, Col de Tende, June 1868, *Burnat s.n.* (FI!; syntype); Pâturages au mont Piernaude pres le col de Tende, 29 June1861, *E. Borgeau 346* (G-BU barcode G00096148!, lectotype; FI!, G barcodes G00169894!, G00169896!, G00169897!, isolectotypes). **Italy** – Liguria – Alpi marittime – Pian Tendasco, 2100 m, supra Pigna, 5 June 1904, *C. Bicknell s.n.* (FI!); Lombardia – Val Vestino, June 1894, *G. Porta s.n.* (FI!); Val di Vestino in pratis montanis Tombea, sol calcar., June 1873, *G. Porta s.n.* (FI!); Val Vestino in m.te Tombea, in pratia alpinis, sol calcareo, 2700 m, 22 June1884, *G. Porta s.n.* (FI!); In pascuis alpinis vallis Vestino, Tirolia, Austria, 15 June 1886, *P. Porta s.n.* (LY!); Tirol. austr. sumit. mont Tombea, Val Vestino, s.d., *P. Porta s.n.* (FI!); Piemonte – Alpi Marittime – Colle di Tenda, versante nord, 16 June 1991, *Ferrari s.n.* (FI!); Alagna-Valsesia, Alp. Mond., 27 June1872, *Carestia s.n.* (FI!); Riva-Valdobbia in Valsesia, vallone d'Otro, regione alpina, Località Straling (Sud-Est), 2 July 1889, *Rasetti s.n.* (FI!); Val Maira (Alpi Cozie) – Colle di Lamprega, praterie a *Festuca spadicea*, 2300-2250 m, 7 July 1975, *G. Boni s.n.* (FI!); Alagna (Valsesia), Alpe Mond., 11 June 1867, *Carestia s.n.* (FI!); Vallone d'Otro, Località Straling, Valsesia, 12 July 1889, *A. Carestia s.n.* (FI!); Mt. Viso, 20 July 1842, *Grenier s.n.* (P barcode P P00654036);Alagna-Valsesia, Alp. Mond., 27 June 1872, *Carestia s.n.* (RO!); Alpi Marittime – pascoli sassosi a 2200 m sotto la vetta del Bec d'Orel, 18 June 1960, *S. Coll. s.n.* (RO!); Limone, praterie salendo al Colle di Tenda, June 1893, *Ferrari s.n.* (RO!); Cuneo, praterie sopra le Grangie Casale sotto il Colle dell'Argentera, 26 June 1900, *Ferrari s.n.* (RO!); Trentino Alto Adige – Tirol. aust. Judicariis, in pascuis M. Bondol, June 1886, *Porta s.n.* (FI!).

F. moggridgei – **France** – Basses Alpes, prairié près au col de Larche, 1900 m, 1904, *Deuliey s.n.* (FI!); Sopra Nizza, June 1864, *Barla s.n.* (FI!); Alpes de Tende, Mont Authion, prairies, 27 May-8 July 1886, *E. Reverchon s.n.* (FI!); La Lauzanier, prairies a 2000 m, 13 June 1901, *Vidal*, *Derbez* e *Bessand s.n.* (FI!); Mentone, s.d., (K barcode K000524693! ex Herb. Hookerianum 1867); **Italy** – Piemonte – Sommites au dessus de la Chartreuse de Pesio, 6 July 1872, *E. Burnat s.n.* (FI!, G-BU barcodes G00096154!, G00096155!, G00096156!; syntypes); Alpi Marittime, Gias Pari di Val Pesio, 19 June 1890, *C. Bicknell s.n.* (FI!); Alpi Marittime – supra Tenda, Pian Tendasco, 2100 m, 5

June 1904, *C. Bicknell s.n.* (FI!); Alpi Marittime − Vaccarile, 9 June 1946, *Beiluti s.n.* (FI!); Pian delle Forre, 900 m, 24 June1947, *Beiluti s.n.* (FI!); Alpi Marittime − Gias Pari, Val Pesio, 22 June 1890, *Bicknell s.n.* (FI!); Alpes Maritimes, sommites voisiner du Col de Tenda, July 1874, *Beltrandi s.n.* (FI!); Lago del Mongioie, Alpi di Mondovì, 18 June 1894, *Ferrari s.n.* (FI!); Alpi Marittime − Mondovì: sotto la vetta del Mongioie, 18 June 1894, *Ferrari s.n.* (RO!); Ravins situés au dessus de la bergerie de Breglio (Alpes de Breglio), près des neiges fondantes, 23 June 1860, *Canut s.n.* (G-BU barcode G00096151!; syntype); Alpi Marittime − praterie sotto la vetta del Mondolè a ponente, Mondovì 11 June 1897, *Ferrari s.n.* (RO!); Alpi Marittime − Gias Pari, Val Pesio, 22 June 1890, *Bicknell s.n.* (RO!); Alpi Marittime − Val Pesio, 22 June 1890, *Bicknell s.n.* (RO!); Limone, Colle di Tenda, 14 May1961, *N. Corradini s.n.* (RO!).

Fritillaria tubaeformis s.str. − **France** − Alpes Maritimes − col de Trente Souches, 22 June 1889, *Vidal s.n.* (FI!); Basses Alpes, col de Vergous, 20 May1885, *Reverchon s.n.* (FI!); Hautes Alpes, Col de Glaize pres Gap, 1800 m, June 1898, *Girod s.n.* (FI!); Hautes Alpes, Charance pres Gap, 9 May 1869, *Burle s.n.* (FI!); Hautes Alpes, Seuse pres Gap, pelouses au bas de la corniche, 1900 m, 31 June 1898, *Faure s.n.* (FI!); Le Devez pres Rabou, Hautes Alpes, 27 May 1866, *A. Burle s.n.* (FI!); Gap a Seuse, pelouses, 1800 m, 31 May 1898, *A. Faure s.n.* (FI!); Chancelaye, prairies a 1800 m, 24 May 1901, *Vidal*, *Derbez* et *Bessan s.n.* (FI!); M. Seuse pres Gap, 1854, *Grenier s.n.* (Herb. J. Gay) (K!; lectotype); Prairie de Fays au Mont Séuse près de Gap (Hautes-Alpes), 2000 mètres d'altitude, 23 June 1858-fin August 1858 (LY!); Alpi Marittime francesi − Montagne de Lachens, margini di pascoli cacuminali, 1700 m, 29 April 2007, *L. Peruzzi* et *K. F. Caparelli s.n.* (PI!); Basses Alpes, col de Vergous, 20 May 1885, *E. Reverchon s.n.* (RO!); Seuse (Hautes-Alpes), June 1861, *Burle s.n.* (LY!); Lusette en Luz (Drome), 1847, *Boullu s.n.* (P barcode P00654037!; lectotype); L'Audibergue, rocailles herbeuses, 1400 m, Andon, 11 May 1969, *s. coll. s.n.* (FI!).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Legends For Figures

Fig. 1 Geographical distribution of *Fritillaria tubaeformis* complex in the Alps (shaded inset, right). Occurrence points of *F. tubaeformis* (white triangles), *F. moggridgei* (yellow circles) and *F. burnatii* (red diamonds) are based on actual distribution and bibliographic records (for France: SILENE, 2015 - Conservatoire Botanique National Méditerranéen, Conservatoire Botanique National Alpin)

Fig. 2 Box plots of simple statistics (line = median**,** empty square = mean of the group**,** bottom-top box = 25-75th percentiles**,** whiskers = maximum and minimum values) of six morphological characters analysed in vivo in *F. tubaeformis* species complex. Abbreviations of variables are: MaxOTL maximum outer tepal length (**a**), MaxITL**,** maximum inner tepal length (**b**), MaxITW, maximum inner tepal width (**c**), MaxOTW**,** maximum outer tepal width (**d**), MaxONL**,** maximum length of outer tepal nectary (**e**), MaxINL**,** maximum length of inner tepal nectary (**f**). All metric characters are expressed in mm

Fig. 3 Lateral view of the flowers in *F. tubaeformis* species complex from specimens of the three type localities. Variability in shape and colour pattern. *Fritillaria burnatii* (**a**), *Fritillaria moggridgei* (**b**), *Fritillaria tubaeformis* (**c**). Metric bar = 2 cm. Red dashed arrows = inner tepals; Black dashed arrows = outer tepals

Fig. 4 Box plots of simple statistics of the width of the largest leaf (MaxLW in mm) in *F. tubaeformis* species complex. Plotted statistics as in Fig. 2

Fig. 5 Mean values of the nine variant characters common to the combined data set (herbarium and in vivo specimens). Results of two-way ANOVA at 0.05 significance level are also given (***$p \leq 0.001$; **$p \leq 0.05$; NS=not statistically different). OTL,

average outer tepal length. OTW, average outer tepal width. ITL, average inner tepal length. ITW, average inner tepal width. MaxLW, width of the largest leaf. LN, total leaf number. LalterN, number of basal alternate leaves. PedL, flower pedicel length

Fig. 6. Principal component analysis (PCA) based on 16 morphological characters of different samples of *F. tubaeformis* species complex. The first two axes explain 69.28% and 30.72% of total variation, respectively.

Fig. 7 Scatterplots of the first and second canonical discriminant functions explaining 72.8 % and 27.2 % of the interspecific taxonomic variation of *F. tubaeformis* species complex. Plot of the individuals, full grey circles indicate the respective group centroids (**a**). Plot of the 16 variables (pin vectors), in the plane of the first two discriminant functions (**b**). Red pins = characters measured in vivo; blue pins = characters derived from herbarium specimens. For explanation of acronyms see Table 1.

Table 1 Morphologic characters used for the morphometric analyses of the *F. tubaeformis* species complex.

Characters

Plant height (mm) [a,b]

MinOTL - minimum outer tepal length (mm) [a]

MaxOTL - maximum outer tepal length (mm) [a]

MinOTW - minimum outer tepal width (mm) [a]

MaxOTW - maximum outer tepal width (mm) [a]

MinITL- minimum inner tepal length (mm) [a]

MaxITL - maximum inner tepal length (mm) [a]

MinITW - minimum inner tepal width (mm) [a]

MaxITW - maximum inner tepal width (mm) [a]

FN - flower number [a]

PedL - pedicel length (mm) [a]

MaxLW - width of the largest basal leaf (mm) [a]

LN - leaf number [a]

LalterN - number of basal altern leaves [a]

LbractN- number of bracteal leaves [a]

Ovary - ovary length (mm) [a]

Style - style length (mm) [a]

Stigma - stigma length (mm) [a]

Filament - stamen filament length (mm) [a]

Anther - anther length (mm) [a]

MinONL - minimum length of outer tepal nectary (mm) [a]

MaxONL - maximum length of outer tepal nectary (mm) [a]

MinONW - minimum width of outer tepal nectary (mm) [a]

MaxONW - maximum width of outer tepal nectary (mm) [a]

MinINL - minimum length of inner tepal nectary (mm) [a]

MaxINL - maximum length of inner tepal nectary (mm) [a]

MinINW - minimum width of inner tepal nectary (mm) [a]

MaxINW - maximum width of inner tepal nectary [a]

OTL_herb - outer tepal length (mm) [b]

OTW_herb - outer tepal width (mm) [b]

ITL_herb - inner tepal length (mm) [b]
ITW_herb - inner tepal width (mm) [b]
FN_herb - flower number [b]
PedL_herb - pedicel length (mm) [b]
MaxLW_herb - width of the largest basal leaf (mm) [b]
LN_herb, leaf number [b]
LalternN_herb, number of basal alternate leaves [b]
LbractN_herb - number of bracteal leaves [b]

[a] characters estimated in vivo; [b] characters estimated in the herbarium specimens.

Table 2 Statistical descriptors of the twenty eight quantitative continuous (24) and cardinal (4) characters estimated in vivo in *F. tubaeformis* species complex. Arithmetical mean (Mean) and standard deviation (SD) are given for each character computed for a priori designated taxa. Results of ANOVA ($p < 0.05$) with *F-test* and *P* values are shown in the last two columns. Characters with *F* value greater than 25.00 and p = 0.000 are given in bold. Letters (a, b, c) denote Tukey HSD pairwise mean comparisons between taxa; taxa with the same letter within a row (variable) do not differ significantly ($p < 0.05$).

Character	*Fritillaria burnatii*			*Fritillaria moggridgei*			*Fritillaria tubaeformis*				
	Sample Size	Mean	SD	Sample Size	Mean	SD	Sample Size	Mean	SD	*F*	*P*
Plant height	50	158.79^a	28.51	21	172.65^a	36.27	15	148.51^a	33.18	2.747	0.070
MinOTL		35.61^a	4.24		41.11^b	3.24		43.10^b	3.56	**28.583**	**0.000**
MaxOTL		37.51^a	3.82		43.02^b	3.18		44.82^b	3.48	**32.353**	**0.000**
MinOTW		12.28^a	2.23		14.34^b	2.11		17.46^c	2.11	**33.854**	**0.000**
MaxOTW		13.73^a	2.31		15.83^b	2.07		19.20^c	1.80	**37.713**	**0.000**
MinITL		35.13^a	4.38		43.07^b	3.72		42.94^b	3.84	**37.930**	**0.000**
MaxITL		37.09^a	4.06		44.78^b	3.85		44.98^b	3.62	**40.797**	**0.000**

MinITW	13.76^a	2.88	22.86^b	2.61	21.63^b	1.91	**108.915**	**0.000**
MaxITW	15.43^a	2.85	24.82^b	2.89	24.36^b	2.57	**111.974**	**0.000**
FN	1.00^a	0.00	1.00^a	0.00	1.00^a	0.00	ND	ND
PedL	20.85^a	8.05	19.85^a	5.54	16.42^a	4.97	2.277	0.109
MaxLW	7.99^a	1.58	16.43^b	5.87	13.94^b	2.44	**76.701**	**0.000**
LN	5.84^a	0.82	5.60^a	0.75	6.33^b	0.49	4.334	0.016
LalternN.	4.84^{ac}	0.82	4.60^a	0.75	5.27^c	0.59	3.424	0.037
LbractN.	1.00^a	0.00	1.05^a	0.22	1.07^a	0.26	1.492	0.231
ovary	7.49^a	1.73	10.37^b	1.46	8.52^a	1.81	21.788	0.000
style	8.03^a	2.08	10.16^b	2.40	10.23^b	3.03	8.813	0.000
stigma	4.75^a	1.53	4.02^a	1.13	2.01^b	0.61	24.662	0.000
filament	12.84^a	1.29	13.00^a	1.94	10.23^b	2.22	16.149	0.000
anther	8.34^a	3.62	12.11^b	2.59	7.22^a	0.98	14.226	0.000
MinONL	6.51^a	1.91	5.24^b	0.94	3.18^c	0.90	**26.349**	**0.000**
MaxONL	8.17^a	1.78	6.89^b	1.22	4.16^c	0.98	**39.162**	**0.000**
MinONW	0.80^a	0.36	0.80^a	0.21	0.79^a	0.17	0.007	0.993
MaxONW	1.11^a	0.42	1.17^a	0.36	1.17^a	0.22	0.261	0.771
MinINL	7.35^a	1.95	6.76^a	1.57	3.32^b	0.72	**32.293**	**0.000**
MaxINL	8.70^a	1.98	7.95^a	1.36	4.33^b	0.39	**39.657**	**0.000**
MinINW	0.83^a	0.49	0.78^a	0.23	0.89^a	0.21	0.735	0.483
MaxINW	1.20^a	0.66	1.07^a	0.27	1.20^a	0.29	0.538	0.586

Table 3 Statistical descriptors of the eleven quantitative continuous (7) and cardinal (4) characters estimated in the herbarium specimens of *F. tubaeformis* species complex. Arithmetical mean (Mean) and standard deviation (SD) are given for each character computed for a priori designated taxa. *F* and *P* values of ANOVA ($p < 0.05$) are shown in the last two columns. Characters with *F* values greater than 15.00 and p = 0.000 are given in bold. Tukey HSD pairwise mean comparisons between taxa; taxa with the same letter within a row (variable) do not differ significantly ($p < 0.05$)

Character	*Fritillaria burnatii*			*Fritillaria moggridgei*			*Fritillaria tubaeformis*			*F*	*P value*
	Sample Size	Mean	SD	Sample Size	Mean	SD	Sample Size	Mean	SD		
Plant heigth	47	191.13^a	36.22	41	186.10^a	47.55	28	207.36^a	55.98	1.893	0.155
OTL_herb		33.86^a	3.19		38.08^b	4.29		39.78^b	5.77	**19.373**	**0.000**
OTW_herb		10.20^a	1.14		10.27^a	1.32		14.38^b	3.24	**47.565**	**0.000**
ITL_herb		33.75^a	3.46		38.87^b	4.50		39.93^b	5.52	**22.745**	**0.000**
ITW_herb		12.30^a	1.82		15.34^b	2.41		19.12^c	3.60	**63.114**	**0.000**
FN_herb		1.00^a	0.00		1.00^a	0.00		1.00^a	0.00	--	--
PedL_herb		20.04^a	7.99		16.64^a	5.60		19.29^a	5.77	2.961	0.056
MaxLW_herb		4.47^a	1.53		9.12^b	3.32		9.05^b	2.92	**43.336**	**0.000**
LN_herb		5.15^a	0.78		5.44^{ab}	0.98		5.68^b	0.77	3.738	0.027
LalternN_herb		4.15^a	0.78		4.39^a	1.02		4.64^a	0.73	3.154	0.046
LbractN_herb		1.00^a	0.00		1.05^a	0.22		1.04^a	0.19	1.098	0.337

Table 4 Statistical analysis of nine quantitative continuous (7) and ordinal (2) characters estimated in the herbarium and *in vivo* specimens (combined data set) of *F. tubaeformis* species complex. Arithmetical mean (Mean) and standard deviation (SD) are given for each type of data. *F* and *P* values of the two-way ANOVA (*p* < 0.05) are given in the last four columns for differences in means (levels) according to *SPECIMEN TYPE* and *TAXA* factors

Levels	Herbarium measurements			In vivo measurements			Specimen type		Taxa	
	Sample Size	Mean	SD	Sample Size	Mean	SD	*P value*	*F value*	*P value*	*F value*
Plant height	116	193.27	46.00	86	160.38	31.98	0.000	31.397	0.795	0.230
OTL		36.78	4.96		39.20	4.91	0.000	60.324	**0.000**	**82.185**
OTW		11.23	2.60		14.48	3.02	0.000	234.582	**0.000**	**116.506**
ITL		37.05	5.16		39.39	5.57	0.000	61.177	**0.000**	**102.125**
ITW		15.02	3.68		18.34	5.24	0.000	229.967	**0.000**	**241.546**
MaxLW		7.22	3.46		11.03	4.92	0.000	134.957	**0.000**	**103.891**
LalterN		4.35	0.88		4.86	0.79	0.000	15.678	**0.009**	**4.829**
LN		5.38	0.87		5.87	0.78	0.000	15.738	**0.003**	**5.938**
PedL		18.66	6.82		19.83	7.16	0.717	0.131	0.059	2.876

All metric characters are expressed in mm. Plant height; OTL, outer tepal length; OTW, outer tepal width; ITL, inner tepal length; ITW, inner tepal width; MaxLW, width of the largest basal leaf; LN, leaf number; LalterN, number of basal alternate leaves; PedL, pedicel length
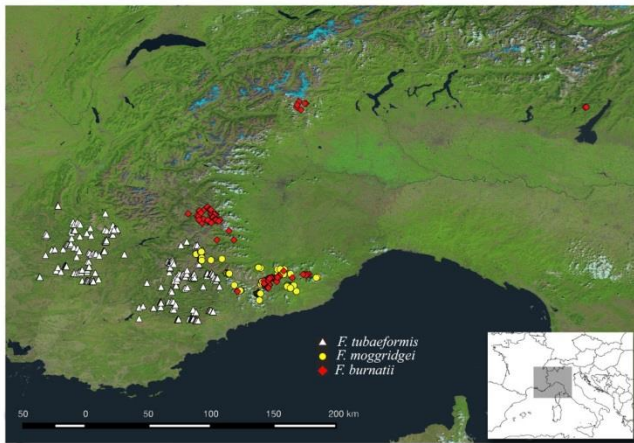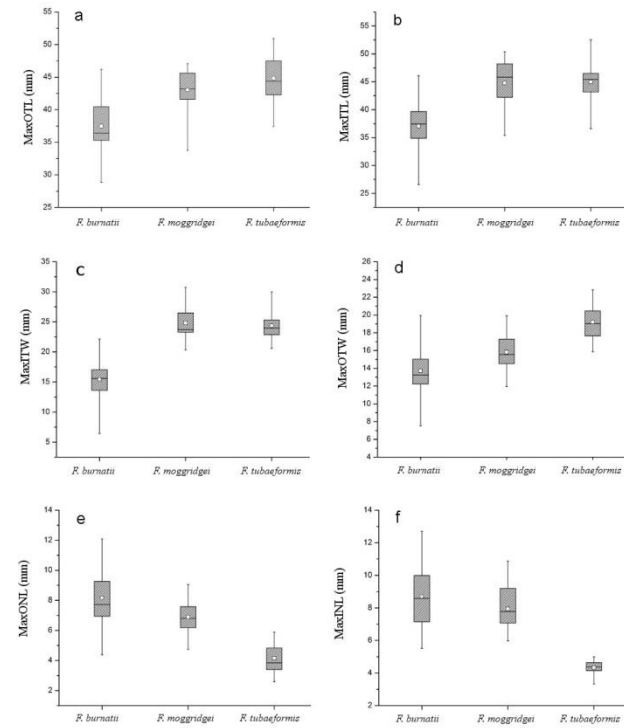
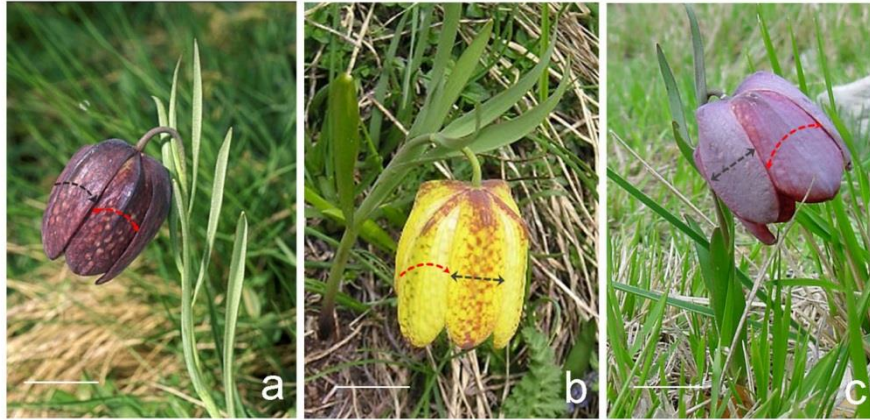Figure 1 Mucciarelli et al. 2016

Figure 2 Mucciarelli et al. 2016

Figure 3 Mucciarelli et al. 2016
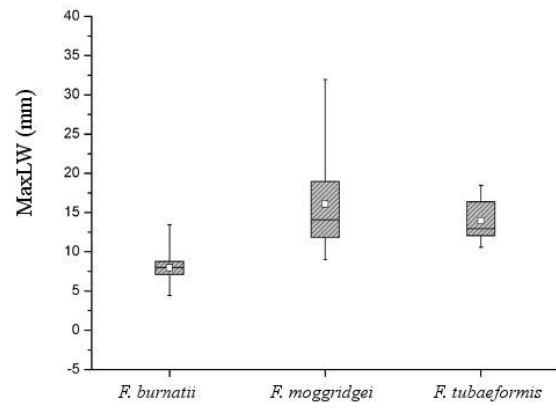
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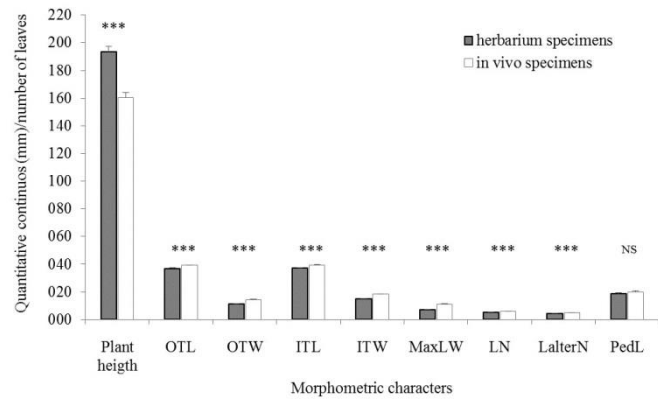
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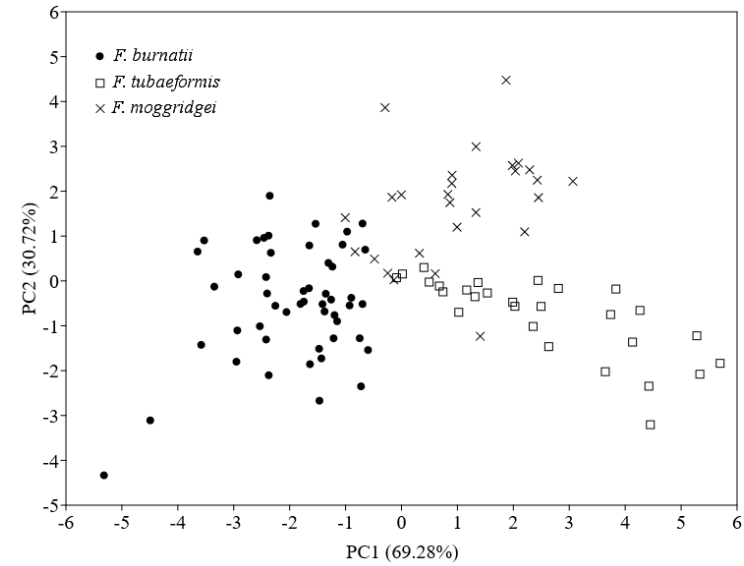
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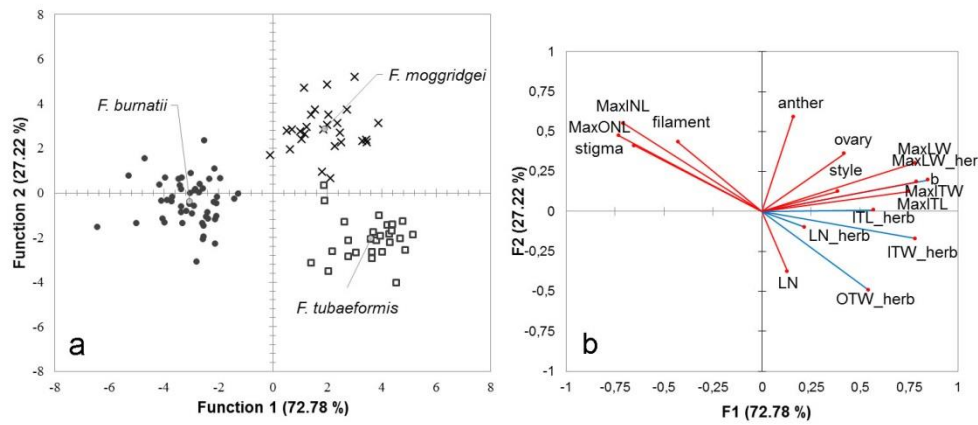
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