Fritillaria messanensis subsp. neglecta (Parl.) Nyman, a fourth subspecies within Fritillaria messanensis Raf. (Liliaceae) from NW Balkans

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Abstract

PERUZZI, L., K. F. CAPARELLI & F. BARTOLUCCI (2009). Fritillaria messanensis subsp. neglecta (Parl.) Nyman, a fourth subspecies within Fritillaria messanensis Raf. (Liliaceae) from NW Balkans. *Candollea* 64: 237-244. In English, English and French abstracts.

Fritillaria neglecta Parl., a critical and neglected species described from Istria, is studied from its *locus classicus* by morphology and karyology. Our results suggest this diploid taxon ($2n = 24$) should be treated at the subspecific rank within *Fritillaria messanensis* Raf. s.l. as a fourth subspecies, endemic to NW Balkans (Croatia: Istria and Dalmatia), showing a peculiar combination of character-states such as, for instance: tepals rounded/spathulate, not upturned at the mouth and with clear fascia; all leaves alternate. A key to the four subspecies of *Fritillaria messanensis* is provided.

Key-words

LILIACEAE – *Fritillaria* – Balkans – Karyology – Karyotype asymmetry – Taxonomy

Résumé

PERUZZI, L., K. F. CAPARELLI & F. BARTOLUCCI (2009). Fritillaria messanensis subsp. neglecta (Parl.) Nyman, une quatrième sous-espèce de Fritillaria messanensis Raf. (Liliaceae) de la région NO des Balkans. *Candollea* 64: 237-244. En anglais, résumés anglais et français.

Fritillaria neglecta Parl., une espèce mal connue décrite d'Istrie, est étudiée de son *locus classicus* par une analyse morphologique et caryologique. Les résultats montrent que ce taxon diploïde ($2n = 24$) doit être considéré comme une sous-espèce de *Fritillaria messanensis* Raf. s.l. de la région NO des Balkans (Croatie: Istrie et Dalmatie), ayant une combinaison particulière de caractères comme des tépales arrondis/spathulés, non retroussés au niveau de leur apex et avec une bande médiane bien marquée, et des feuilles toutes alternes. Une clé de détermination des quatre sous-espèces de *Fritillaria messanensis* est fournie.

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Submitted on February 23, 2009. Accepted on September 24, 2009. Edited by P. Perret

ISSN: 0373-2967 *Candollea* 64(2): 237-244 (2009)

Introduction

Fritillaria L. *(Liliaceae)* includes about 140 species of perennial bulbous geophytes distributed in the Northern Hemisphere, from North America to the Mediterranean region and temperate Asia (Bartolucci & al., 2009). *Fritillaria* species are found in the Mediterranean and Irano-Turanian phytogeographical regions. The genus is represented worldwide by 165 taxa of which 40 have been recorded from Turkey, 25 from Greece, 24 from China, 20 from California, 18 from Iran (Rix, 2001). According to the classification proposed by Rix (2001), based on morphological characters, *Fritillaria* is subdivided into eight subgenera: subgen. *Fritillaria*, [including two sections: *Olostylae* (six series) and *Fritillaria* (ten series)], subgen. *Rhinopetalum* Fisch., subgen. *Japonica* Rix, subgen. *Theresia* K. Koch, subgen. *Petilium* (L.) Endl., subgen. *Liliorhiza* (Kellogg) Benth. & Hook. f. (three series), subgen. *Davidii* Rix and subgen. *Korolkovia* Rix. A recent phylogenetic analysis (Rønsted & al., 2005) shows that *Fritillaria* is monophyletic and indeed supports the infrageneric classification proposed by Rix (2001).

Fritillaria messanensis Raf. belongs to *Fritillaria* sect. *Fritillaria*. This section, comprising more than half of the existing species, is characterized by a bulb with 2 (rarely 3 or 4) fleshy scales, usually covered by a translucent tunic; 1 campanulate tessellated flower, sometimes 2 or 3, rarely more; opposite or alternate leaves. According to Kamari & Phitos (2006), *F. messanensis* consists of three subspecies: subsp. *messanensis*, occurring in Greece (Mt. Olimbos and Peloponnese) and S Italy (Calabria and Sicily), subsp. *gracilis* (Ebel) Rix, occurring in the Ionian Islands, Albania, Serbia and Montenegro (see also Tomović & al., 2007) and subsp. *sphaciotica* (Gand.) Kamari & Phitos, endemic to Kriti. The same authors doubtfully synonymize *F. neglecta* Parl. with *F. messanensis* subsp. *gracilis*, however not mentioning Croatia (Dalmatia and Istria) in its distribution range ("The alleged occurrence of *F. messanensis* in Croatia and Herzegovina needs verification, since all the specimens checked by us do not belong to the taxon"). Accordingly, as recently stated also by Tomović & al. (2007), "there is a certain confusion concerning the presence of *F. messanensis* s.l. in Croatia", as already stated by Lengyel (1931). *Fritillaria neglecta* was described by Parlatore (1857) based on a single herbarium specimen from Istria, which is indeed clearly referable to *F. messanensis* s.l., but does not appear to fit with any of the currently recognized subspecies (Peruzzi & Bartolucci, 2009). The aim of this study is to clarify the taxonomic status and systematic value of this taxon.

Materials and methods

Specimens seen. – **Croatia. Istria:** In Monte Zagor supra Fianonam, oppidum Istriae, rara, 1854, *Tommasini* (FI, lectotype of *Fritillaria neglecta*, see Peruzzi & Bartolucci, 2009); Monte Maggiore, prope Fiume, Istria, 4400', 25.V.1870, *Smith* (FI); Monte Sissol verso Cosliaco, 6.V.1924, *Calgari* (FI); s. loc., III.1946, *s.coll.* (RO); versante NW del Monte Sisol, gariga a 750 m, 27.IV.2007, *L. Peruzzi & K. F. Caparelli* (PI).

Plants in cultivation. – **Croatia. Istria:** Versante NW del Monte Sisol, gariga a 750 m, 27.IV.2007, *L. Peruzzi & K. F. Caparelli* (cult. Hort. Bot. University of Pisa, acc. n. 282-2007). **Dalmatia:** Biokovo, 13.V.2003, *L. Peruzzi & N. G. Passalacqua* (cult. Hort. Bot. of Pisa, acc. n. 461-2006).

Morphological study. – The following characters were measured and/or observed for *F. neglecta* and compared with the data reported by Kamari & Phitos (2006) for the other three subspecies: plant size (cm), plant colour, leaf width (cm), perigon length (cm), tessellation (presence/absence), fascia (presence/absence), tepals shape, number of floral bracts. A total of 30 plants from 2 localities were studied. Since *F. neglecta* appeared very close to *F. messanensis* subsp. *messanensis*, we made deeper morphometric comparisons among these two taxa, based also on specimens listed in Appendix 1, for a total of 41 plants from 4 populations of *F. messanensis* subsp. *messanensis*. The following characters were measured, besides those listed above: basal leaves arrangement, flowers number, leaves number (bracts excluded), outer tepals length (mm), outer tepals width (mm), inner tepals length (mm), inner tepals width (mm), pedicel flowers length (mm), maximum leaves width (mm), ovary length (mm), style length (mm), stigma length (mm), filament length (mm), anthers length (mm), outer tepals nectaries length (mm), outer tepals nectaries width (mm), inner tepals nectaries length (mm), inner tepals nectaries width (mm). The variables were processed with the software of statistical and multivariate analysis Data Desk 6.1.

Karyological study. – Plants were collected *in situ* and karyologically studied, after cultivation in the Botanic Garden of Pisa University. Root tips were pretreated with a 0.3% solution of colchicine and fixed in Carnoy; afterwards they were hydrolyzed in 1N HCl solution and coloured with fuchsine; at the end, they were squashed in acetic orcein for counting and observation of chromosomes. Karyotype formulas and terminology are according to Levan & al. (1964). At least five plates were measured in order to build the idiogram. The graphic method proposed by Plummer & al. (2003) was used to match homologous chromosomes. This involves plotting the relative length of each chromosome [= (length of individual chromosome/total length of all chromosomes) × 100] against its arm ratio, in order to pair chromosomes. For the present analyses two coefficients of variation (CV) were found

to be particularly informative measures of asymmetry (PASZKO, 2006). The CV_{CI} index evaluates differences in centromere position for each chromosome in the karyotype and provides a measure of intrachromosmal asymmetry. In contrast the CV_{CL} gives a measure of interchromosomal asymmetry as it reflects how variable the chromosome sizes are in the karyotype. In both cases, the larger the value the greater the asymmetry in the karyotype. Also, THL (total haploid length of the chromosome complement, in micrometers) was calculated. Karyotype asymmetry values and THLs were calculated also for the other subspecies, from literature data: GORI (1958), KAMARI & PHITOS (2006) and PERUZZI & al. (2009) for subsp. *messanensis;* KAMARI & PHITOS (2006) for subsp. *gracilis* and subsp. *sphaciotica*. Finally, to test a correlation between karyotypic (inferred from CVs and THL) and geographical distances (in Km) among every pair of populations, a Mantel test (computing 1000 permutations) was performed using GenAlEx (PEAKALL & SMOUSE, 2001).

Results

Fritillaria neglecta has a stem (17-)20-36(-44) cm tall and glaucous green. The leaves are 0.3-1(-1.4) cm broad, glaucous green, usually linear to narrowly lanceolate, the uppermost usually alternate. The flowers are campanulate, (2-)2.5-3.4 (-4.7) cm long, usually tessellated, with yellowish-greenish fascia, segments not upturned at the mouth, distinctly rounded-spathulate at apex (Fig. 1).

Our morphological observations are summarized in Table 1. *Fritillaria neglecta* resembles *F. messanensis* subsp. *gracilis* in its small size and leaves usually all alternate, but it is easily distinguished from it for the larger perigon size and the clear presence of fascia. These characters remind closely *F. messanensis* subsp. *messanensis*. However, *F. neglecta* is clearly distinguished also from the latter unit thanks to the different perigon shape and

Fig. 1. – *Fritillaria neglecta* Parl. in flower from its *locus classicus*, Monte Sisol **(A)** and from Biokovo **(B)**. [Photos: L. Peruzzi (A); N. G. Passalacqua (B)]

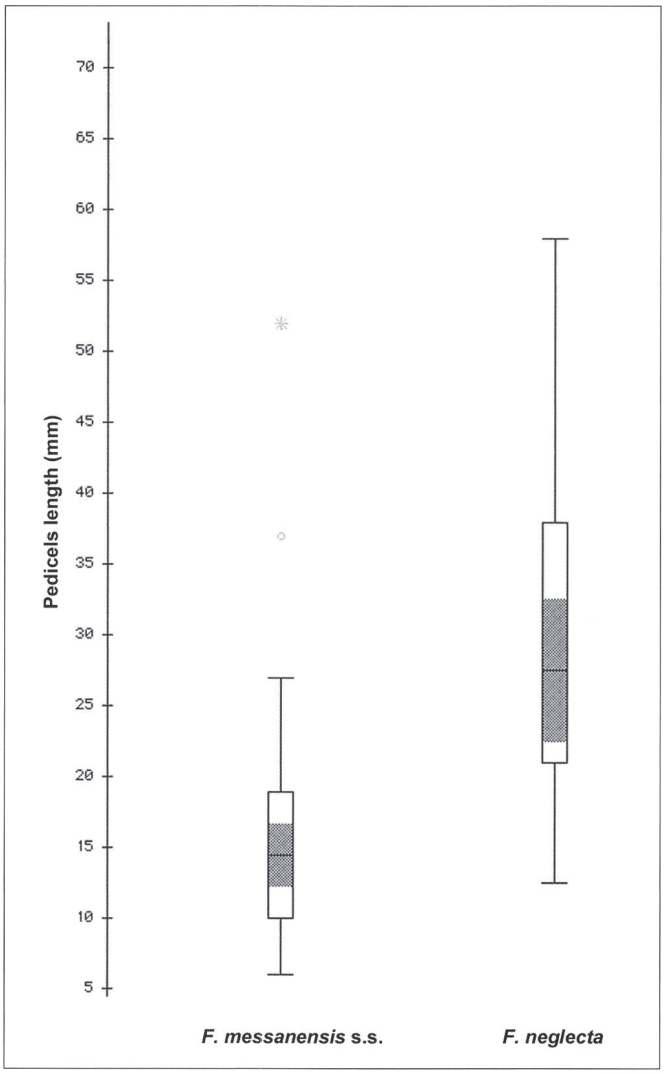

Fig 2. – Boxplots illustrating the variability – in *Fritillaria messanensis* s.s. and *F. neglecta* – of pedicels length. [The outlined central box depicts the middle 50% of the data extending from upper to lower quartile; the horizontal bar is at the median. The ends of the vertical lines (or "whiskers") indicate the minimum and maximum data values, unless outliers are present in which case the whiskers extend to a maximum of 1.5 times the inter-quartile range. Superimposed grey areas indicate confidence interval bounds around its median (median ± 1.58 times the inter-quartile range). Circles indicate outliers, unless extreme outliers are present in which case the circles extend to a maximum of 3 times the inter-quartile range and the extreme outliers are indicated as asterisks]

also from other features such as: the larger pedicels (Fig. 2), the minor length of perigon and nectaries, especially of the inner tepals (Fig. 3-4). The other characters surveyed did not result of particular significance (data not shown).

As far as karyology is concerned, *F. neglecta* is a diploid taxon with $2n = 24$ chromosomes in material from its *locus classicus*. Karyotype features are summarized in Table 2. *Fritillaria neglecta* appears karyologically closer to *F. messanensis* subsp. *gracilis* (Fig. 5), showing the highest CV_{CI} value

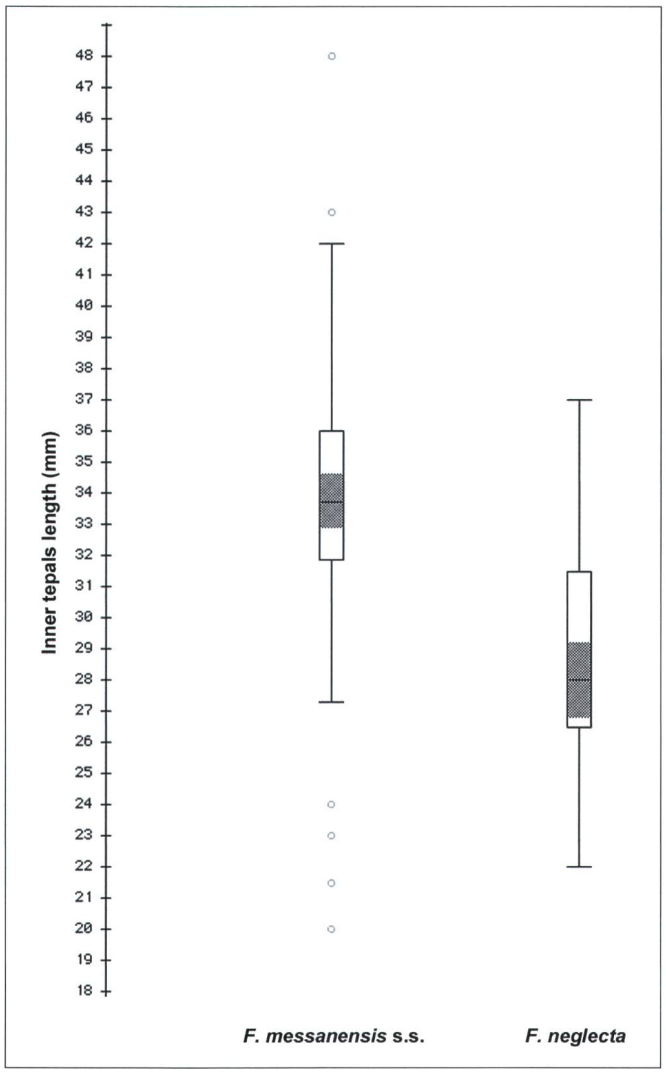

Fig. 3. – Boxplots illustrating the variability – in *Fritillaria messanensis* s.s. and *F. neglecta* – of inner tepals length. [See legend of Fig. 2 for more explanations]

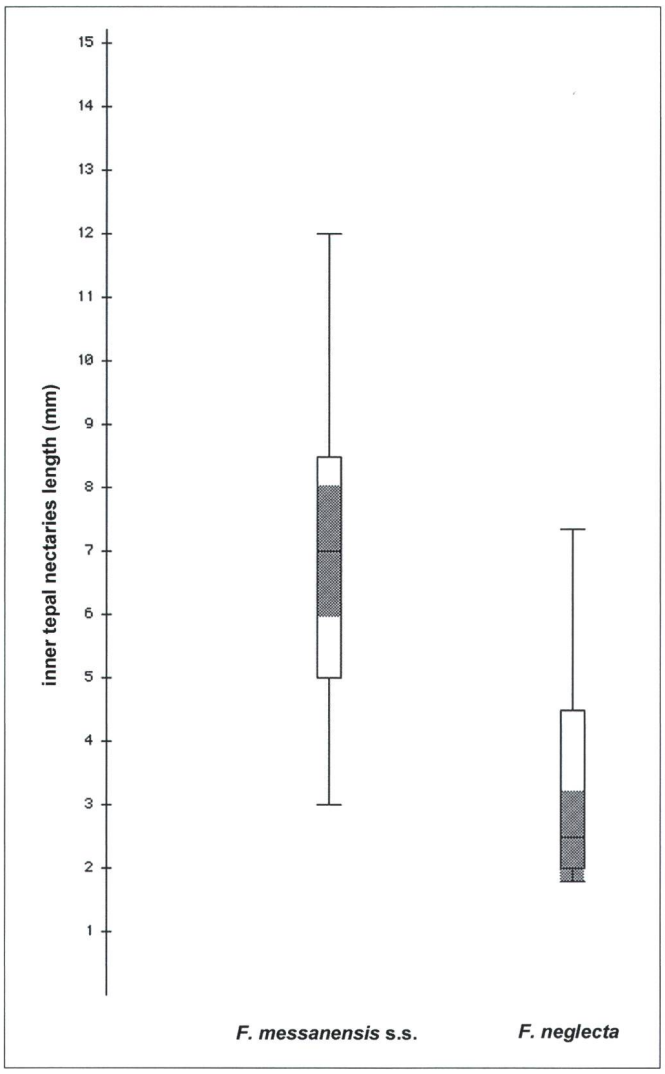

Fig. 4. – Boxplots illustrating the variability – in *Fritillaria messanensis* s.s. and *F. neglecta* – of inner tepal nectaries length. [See legend of Fig. 2 for more explanations]

and a high CV_{CL} value respect to other *F. messanensis* s.l. accessions. Finally, no significant correlation was found between karyotypic and geographic distance, based on Mantel test ($r = 0.026$, $P = 0.3$).

Discussion

According to our results, *F. neglecta* clearly falls within the variability of *F. messanensis* s.l., even if it does not fit with any of the currently recognized subspecies on morphologicalgrounds. The most similar unit seems to be the subsp. *messanensis*, which however is clearly distinguished thanks to several character-states. The diploid level of *F. neglecta*, with $2n = 24$ chromosomes, is shared with *F. messanensis* s.l. and many other species of the genus *Fritillaria* (Peruzzi & al., 2009). On the other hand, a karyotype feature distinguishes *F. neglecta* from *F. messanensis* s.l., falling somewhat close to one *F. messanensis* subsp. *gracilis* accession only. The Mantel test failed to find a correlation between karyological and geographical distance (i.e. a genocline) and this possibly means that the separation of the taxa is still not complete from a genetic point of view. This confirms and supports the taxonomic rank of subspecies for all the taxa involved (and allopatric), including *F. neglecta*. The combination *F. messanensis* subsp. *neglecta* (Parl.) Nyman is available, and is here re-used. The latter subspecies appears endemic of coastal mountains of Croatia (Dalmatia and Istria). An updated identification key for the subspecies of *F. messanensis* follows.

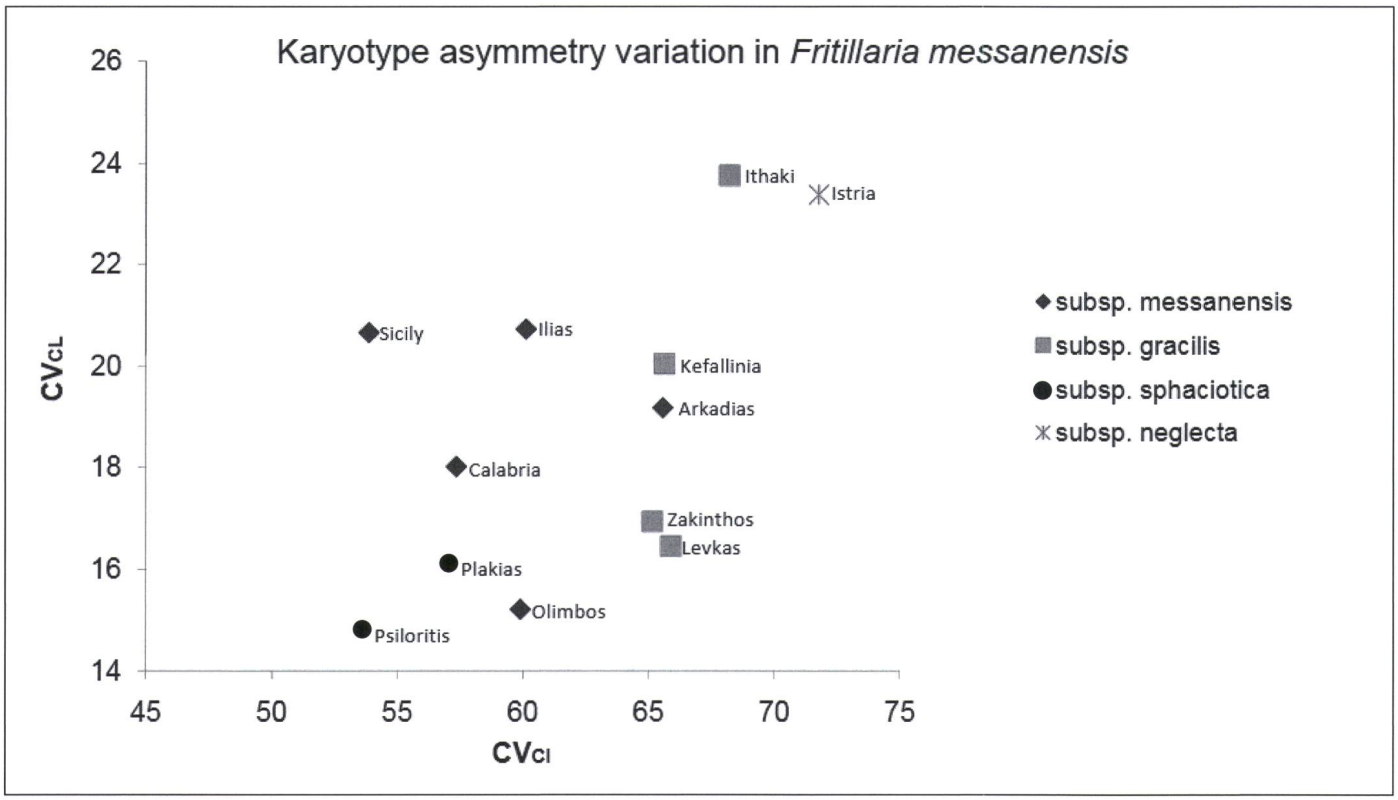

Fig. 5. – Scatter plot showing karyotype asymmetry variation in *Fritillaria messanensis* Raf. s.l. CV_{CI} is represented in the x axis, CV_{CL} in the y axis.

1. Flowers with unclear or without fascia..... ..... subsp. ***gracilis*** (Ebel) Rix
1a. Flowers with clear yellowish/greenish fascia 2

2. All leaves (including floral bracts) usually alternate; tepals rounded/spathulate, not upturned at the mouth..... ..... subsp. ***neglecta*** (Parl.) Nyman
2a. Floral bracts usually in a whorl of 3; tepals acute/subacute, slightly upturned at the mouth 3

3. Plant glaucous green; stem 20-70(-110) cm tall, leaves (0.5-) 0.7-1(-1.4) cm broad ..... subsp. ***messanensis***
3a. Plant purplish glaucous-green and often purple at the base; stem 10-20(-25) cm tall, leaves 0.2-0.4(-0.6) cm broad .... subsp. ***sphaciotica*** (Gand.) Kamari & Phitos

Taxonomic setting

Fritillaria messanensis subsp. ***neglecta*** (Parl.) Nyman, Consp. Fl. Eur.: 721. 1882.

≡ *Fritillaria neglecta* Parl., Fl. Ital. 2: 415. 1857.

Lectotype (designated by Peruzzi & Bartolucci, 2009): "In Monte Zagor supra Fianonam, oppidum Istriae, rara", 1854, *Tommasini s.n.* (FI!).

Iconography. – See Fig. 1. Reichenbach (1848, sub *F. messanensis*) published a plate of a specimen from "Istria", which perfectly fits with *F. neglecta* (Fig. 6). This icon is cited by Parlatore (1857) in the protologue of this latter name and therefore is original material for it.

Distribution. – Coastal mountains of NW Balkans (Croatia: Istria and Dalmatia).

Fig. 6. – Plate of *Fritillaria messanensis* Raf. from Istria (at the centre) published by REICHENBACH (1848: tab. CCCCXLV), which is also original material for *F. neglecta* Parl.

Acknowledgements

The authors wish to thank for laboratory help: Mrs Lisa Savallo, Mr Antonio Giordani. Financial funding (EX60%) from University of Pisa is gratefully acknowledged.

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Appendix 1. – Specimens seen of *Fritillaria messanensis* Raf. subsp. *messanensis*.

Italy. Sicily: Colli presso Messina, 26.II.1940, *V. Bambacioni* (RO); Messina, s.d., *F. Cortesi* (RO); In herbidis collinis: Messina ad Gravitelli, II.1880, *s.coll.* (RO); Messina, 1883, *s.coll.* (RO); Messina, copiosa in arvis Oleae satis loco Gravitelli dicto in possessione, 14.V.1923, *E. Mangano* (FI, RO); Gravitelli, Messina, II.1950, *Gori* (FI); Nei luoghi boschivi aprici, a Scirpi, III, *Nicotra* (FI). **Calabria:** Dintorni di Catanzaro, 23.III.1883, *Fiori* (FI); Corigliano, 1851, *s.coll.* (FI); Tarsia (prov. di Cosenza), bivio dopo il lago (direz. Rossano) sulla dx, ca. 80 m, 7.IV.2007, *L. Peruzzi* (PI).

Table 1. – Comparison of morphological features among the subspecies of *Fritillaria messanensis* Raf.

	subsp. ***gracilis***	subsp. ***messanensis***	subsp. ***neglecta***	subsp. ***sphaciotica***
Plant size [cm]	up to 60	20-70(-110)	(17-)20-36(-44)	10-20(-25)
Plant colour	glaucous-green	glaucous-green	glaucous-green	purplish green
Leaves width [cm]	(0.4-)0.6-0.8(-1)	(0.5-)0.7-1(-1.4)	0.3-1(-1.4)	0.2-0.4(-0.6)
Perigon length [cm]	2.2-2.8(-3.3)	3-4(-4.5)	(2-)2.5-3.4(-4.7)	(2.5-)2.8-3.5
Tessellation	no/obscure	yes	yes	no/obscure
Fascia	no	yes	yes	yes
Tepals shape	acute/subacute distinctly upturned at the mouth	acute-subacute slightly upturned at the mouth	rounded/spathulate not upturned at the mouth	subacute slightly upturned at the mouth
Floral bracts	usually single	usually in a whorl of 3	usually single	usually in a whorl of 3

Table 2. – Karyotype features of *Fritillaria neglecta* Parl. compared with data derived from previous works on *F. messanensis* Raf. s.l. A = KAMARI & PHITOS, 2006; B = GORI, 1958; C = PERUZZI & al., 2009.

subspecies	locality	CV_{CI}	CV_{CL}	THL	t	st	sm	m	source of data
gracilis	Kefallinia (SW Greece)	65.63	20.05	208.1	7	3	2	/	A
gracilis	Ithaki (SW Greece)	68.21	23.76	177.4	6	4	1	1	A
gracilis	Levkas (SW Greece)	65.14	16.94	195.4	7	3	1	1	A
gracilis	Zakinthos (SW Greece)	65.88	16.45	175.7	5	5	1	1	A
messanensis	Messina (Sicily)	53.87	20.66	98.74	4	6	2	/	B
messanensis	Stilo (S Italy)	57.35	18.02	101.9	3	7	/	2	C
messanensis	Olimbos (NE Greece)	59.9	15.21	183	6	4	1	1	A
messanensis	Arkadia (S Greece)	65.56	19.18	170.8	7	2	2	1	A
messanensis	Ilias (S Greece)	60.12	20.73	127.1	4	4	1	1	A
neglecta	Istria (N Croatia)	71.76	23.38	185.1	6	3	2	1	present study
sphaciotica	Plakias (Kriti)	56.76	16.07	175.1	6	5	1	/	A
sphaciotica	Psiloritis (Kriti)	53.44	14.8	170.2	3	6	2	1	A

[**Abbreviations:** **CV_{CI}** = coefficient of variation of centromeric index; **CV_{CL}** = coefficient of variation of chromosome length (PASZKO, 2006); **THL** = total haploid length of chromosome complement, in µm; number of chromosomes of terminal **(t)**, sub-terminal **(st)**, sub-median **(sm)** and median **(m)** type (LEVAN & al., 1964)]