

Prilozi poznavanju flore Hrvatske / Contributions to the knowledge of the Croatian flora

Curators in action: intricate genus *Fritillaria* L. (Liliaceae) from ZA, ZAHO, CNHM and ZAGR revised and digitized

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Abstract

A total of 289 herbarium sheets with 803 specimens from the genus *Fritillaria* within ZA, ZAHO, CNHM and ZAGR collections were digitized. Altogether 23 taxa were registered within studied collections. The exsiccata originate from 20, mostly European countries; however 72% are from Croatia. The revision of the herbarium sheets collected in

Croatia resulted with five native taxa and their historical distributional data for the country. The average age of the collected specimens was 85 years. Based on our herbaria revision, the area of distribution of *F. messanensis* ssp. *gracilis* greatly increased.

Keywords: digitization, distribution, Flora Croatica Database, *Fritillaria*, herbarium revision

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Sažetak

Digitalizacijom roda *Fritillaria* (kockavica) u herbarijskim zbirkama ZA, ZAHO, CNHM i ZAGR obuhvaćeno je ukupno 289 herbarijskih listova s 803 jedinke. Zabilježene su ukupno 23 svojte. Eksikati potječu iz 20 uglavnom europskih država, od čega 72 % iz Hrvatske. Revizijom herbarijskih listova

sakupljenih na području Hrvatske zabilježeno je pet autohtonih svojti kockavica te su dobiveni povijesni podaci o njihovoj rasprostranjenosti. Prosječna starost herbarijskih primjeraka je 85 godina. Revizijom herbarijskih primjeraka areal svojte *F. messanensis* ssp. *gracilis* je značajno povećan.

Ključne riječi: digitalizacija, Flora Croatica Database, *Fritillaria*, herbarijska revizija, rasprostranjenost

Introduction

For a long period, there were many ambiguities and confusions in identifying taxa of the genus *Fritillaria* in Croatia, essentially due to numerous synonyms and inadequate identification keys, causing major problems (Kranjčev & Šešok 2016). Therefore, a systematic and comprehensive study into this genus was recently performed, based exclusively on field data and morphometrics of living material (Kranjčev & Šešok 2016). The study revealed the existence of five taxa for the Croatian territory: *F. graeca* Boiss. et Spruner ssp. *thessala* (Boiss.) Rix, *F. meleagris* L., *F. messanensis* Raf. ssp. *gracilis* (Ebel) Rix, *F. messanensis* Raf. ssp. *neglecta* (Parl.) Nyman and *F. montana* Hoppe ex W.D.J.Koch (Kranjčev & Šešok 2016). At the end of that study, the curators of the ZA, ZAHO, CNHM and ZAGR were kindly asked to revise exsiccates from their collections, and the acquired data were published as *specimina visa* in Kranjčev & Šešok (2016). Since herbarium data were not discussed nor analysed in detail by Kranjčev & Šešok (2016), the aim of this article is to present: (i) an update of the distribution data based on herbarium data, (ii) taxa analysis in terms of spatial distribution, collectors, collecting dates and phenophases at collecting time and (iii) comparison and harmonisation of ongoing digitization practices present in different Croatian collections.

Currently, Croatia has eleven officially registered herbarium collections (Thiers 2017) of which ZA, ZAHO and CNHM are the largest ones (Vrbek 1999, Horvat & Plazibat 2007). The herbarium ZA is the oldest and largest in Croatia, founded in 1880, with an estimated 180.000 specimens. The herbarium ZAHO was established in 1983 and keeps ca. 78.000 specimens (Horvat & Plazibat 2007). The herbarium CNHM was founded in 1987 and consists of ca. 100.000 specimens. The Herbarium ZAGR is one of the youngest herbaria in Croatia, established in 2013 with estimated 7.500 to 10.000 specimens (Bogdanović 2013, Bogdanović et al. 2016). All above mentioned herbaria started digitization processes in recent years (CNHM in 2008, ZA, ZAHO and ZAGR in 2015) after the purchase of equipment, extensive testing and technical improvements. The large collection of orchids was chosen for initial digitization testing in ZA (Stančić et al. 2016). Publically accessible Virtual Herbarium databases were designed for CNHM and ZAGR, while during 2017 a similar website is planned for ZA and ZAHO.

Material and methods

The herbarium specimens of the genus *Fritillaria* (Liliaceae) were searched for within four Croatian herbaria (ZA, ZAHO, CNHM and ZAGR, acronyms are according to Thiers 2017) and used for the purpose of detailed identification and digitization.

Revision of the specimens collected in Croatia and neighbouring countries was done according to the most recent identification key for Croatian taxa (Kranjčev & Šešok 2016), based mostly on flower characters and developed through extensive field research of populations in Croatia. Partial damaging of the dried pressed flowers could not be avoided, since the nectaria had to be carefully checked in order to identify some taxa. Sectioned flowers were, except in CNHM, preserved and mounted on herbarium sheets along with complete ones, but in a way that the inner surface of the tepala with the nectarium could be easily seen. The specimens in fruit could not be identified with certainty; therefore they remained labelled as *Fritillaria* sp. For the specimens in fruits previously identified by the collectors, we left the name; however, those records should be treated with caution.

Since herbarium sheets in the ZA and ZAHO collections were not in a condition allowing easy digitization, e.g. the sheet format was too large for the common A3 scanner and the plant material was not mounted, a pre-digitization process had to be performed first. This process included gentle removal of insecticide powder using forceps and fine brushes, and translocation of plant material to new herbarium sheet format (42.5 x 29 cm) with dimensions adequate for scanning (Fig. 1). Insecticide powder was in use up to the 2000s, after which it has been removed on several occasions. Currently it is present in ZAHO and often within ZA *Herbarium generale* (collection formed via exchange with other world herbaria). After the plant material was translocated, mounting with pH neutral adhesive tape on herbarium sheets and the gluing of herbarium labels with Gaylord pH neutral white adhesive took place. The latter steps are regular procedure within the ZAGR collection as well (Bogdanović et al. 2016). In the CNHM, the plants were not mounted, mostly due to usage of photographic instead of scanning equipment. However, plants from the *Herbarium Trinajstić* within CNHM collection were originally mounted with adhesive tape by collector. After the cleaning, herbarium sheets were placed on the new acid free paper. A stamp with the herbarium ID (ZA, ZAHO, ZAGR), colour plate and ruler were added to each sheet prior to scanning or photographing.



Figure 1. Pre-digitization process in ZAHO collection: a, b) removal of insecticide powder, c, d) mounting of *Fritillaria* specimens and label(s).

The original metadata from all *Fritillaria* herbarium sheets: herbarium ID, inventory number (CNHM), taxon name, locality, habitat, geographical coordinates, collector(s) and identifier(s), collecting dates and collection number were stored consecutively within the Flora Croatica Database. The full transcription of the original label's text, accompanied by an up-to-date interpretation of collection locality was provided. This was a useful starting point in preparation of as accurate as possible distribution maps for all the taxa within the GIS environment (ESRI 2013). In addition, the number of specimens on each herbarium sheet was counted; moreover, the number of blooming individuals as well as those in fruit was carefully noted.

Finally, the prepared sheets were scanned (ZA, ZAHO, ZAGR) or photographed (CNHM) using available scanning or photography equipment (Fig. 2). Generally, the scanners are considered less adequate for herbarium digitization, because they require inverse placement of the specimens,

resulting in damage and loss of material (Berendsohn et al. 2005). In order to avoid that, in the ZA and ZAHO collections a special electric mobile stalk for inversed scanning has been designed and constructed, enabling the safe handling with sensitive plant material.

The entire herbarium sheets of *Fritillaria* specimens were scanned or photographed with the image resolution sufficiently high to show details of the plants and label texts. Those high resolution images were uploaded in Flora Croatica Database (Nikolić 2017), and are accessible in ZAGR Virtual Herbarium database¹ and CNHM Virtual herbarium database² as well.

In addition, all available photographs in the Flora Croatica Database Gallery were carefully examined and revised, excluding photographs of the plants in fruit. The updates within the database were done in cooperation and with permission of the authors of the photographs.



Figure 2. Digitization equipment: a) inversed Epson Expression 11000XL Pro A3 scanner (ZA & ZAHO), b) Microtek ScanMaker 9800 XY Plus scanner (ZAGR), c) DSLR digital camera Canon EOS Digital Rebel XSi 450D with Canon 18 - 55 mm focal length (CNHM).

¹ <http://herbarium.agr.hr/>

² <http://www.hpm.hr/Odjeli%20i%20zbirke/Botani%C4%8Dki%20odjel/CNHM%20-%20Virtualni%20herbarij>

Results and discussion

In total, 289 herbarium sheets were found within four studied collections. Almost 80% of the herbarium sheets are stored within the ZAHO and ZA collections, followed by CNHM (21%) and ZAGR (1%) collections (Fig. 3). In most cases, several specimens were stored per sole herbarium sheet, the most common numbers being three (35%) and two (24%) (Fig. 4). Altogether, 803 specimens were collected and stored in studied herbaria.

A *make-over* during the pre-digitization process was performed on all the herbarium specimens (224) from ZA and ZAHO and partly from CNHM (*Herbarium Trinajstić*), mostly old and in poor condition (Fig. 5).

There are 23 taxa found within studied collection, five of them being native in Croatia. The taxonomic

revision which included the specimens collected in Croatia, revealed the existence of five native and one ornamental *Fritillaria* taxa in Croatia, among which *F. meleagris* occurred on the largest number of the herbarium sheets (88), followed by *F. messanensis* ssp. *neglecta* (63) and *F. montana* (47) (Tab. 1). The other two native taxa (*F. messanensis* ssp. *gracilis* and *F. graeca* ssp. *thessala*) were collected rarely. For ten specimens it was not possible to identify the species rank, mostly due to the lack of flowers. The highest number of *Fritillaria* taxa was found in ZA collection (23, with all 5 native Croatian taxa included), followed by CNHM (3), ZAHO (2) and ZAGR (1), all with only native taxa from Croatia.

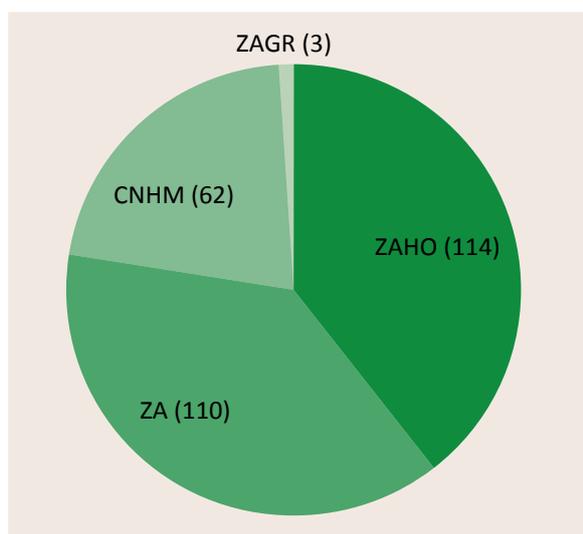


Figure 3. The number of herbarium sheets with *Fritillaria* specimens stored in studied collections (ZA - Herbarium Croaticum, ZAHO - Herbarium of Ivo and Marija Horvat, CNHM - Herbarium of Croatian Natural History Museum, ZAGR - Herbarium of the Faculty of Agriculture, University of Zagreb).

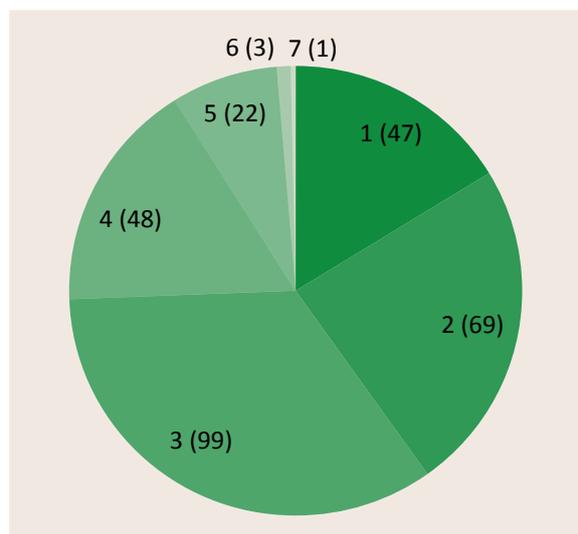


Figure 4. The number of specimens stored on a sole herbarium sheet. Numbers of herbarium sheets are shown in brackets.

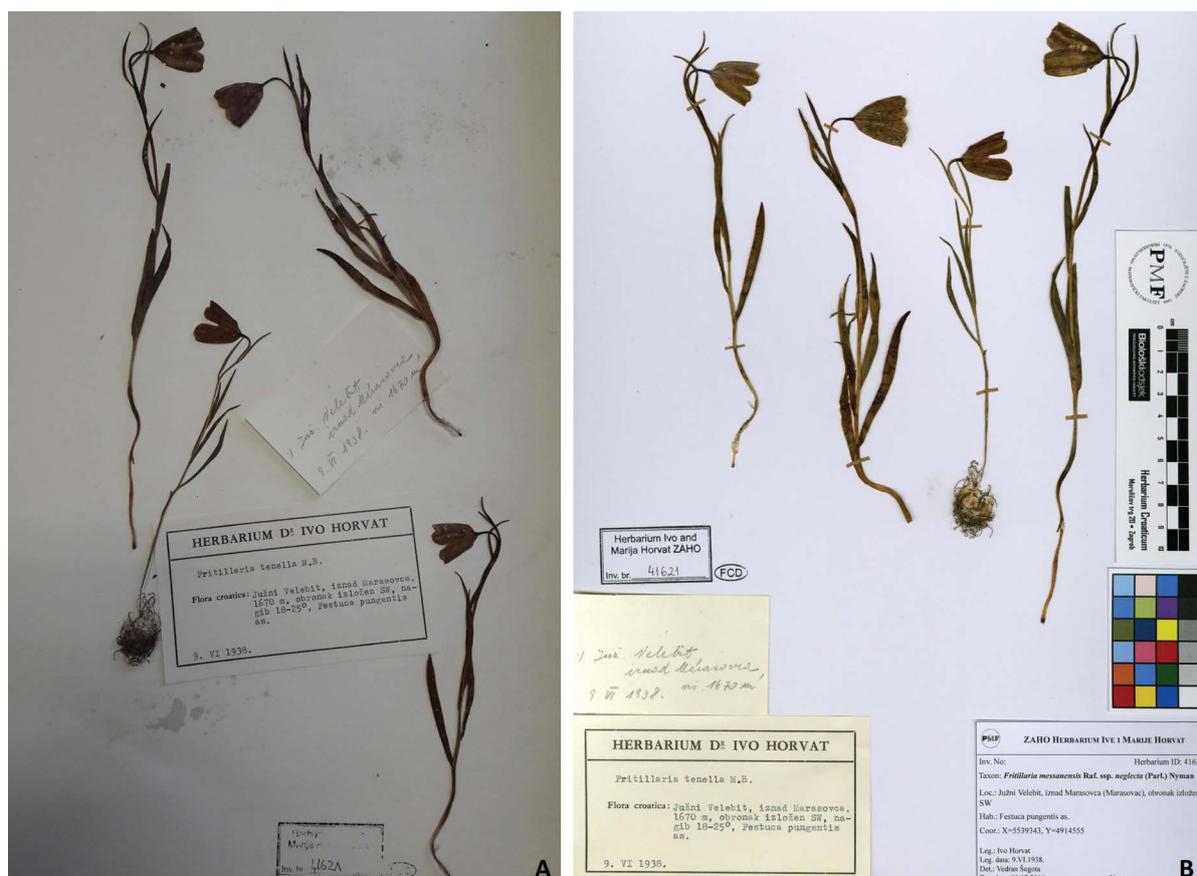


Figure 5. Herbarium sheet of *Fritillaria messanensis* Raf. ssp. *neglecta* (Parl.) Nyman (ZAH041621) before (A) and after (B) the restoration, revision and digital imaging.

Table 1. The number of herbarium sheets for each taxon across the studied collections. The taxa native in Croatia are bolded.

Taxa	Total	ZAHO	ZA	CNHM	ZAGR
<i>Fritillaria bithynica</i> Baker	1	-	1	-	-
<i>Fritillaria collina</i> Adams	2	-	2	-	-
<i>Fritillaria conica</i> Boiss.	1	-	1	-	-
<i>Fritillaria ehrhartii</i> Boiss. et Orph.	1	-	1	-	-
<i>Fritillaria graeca</i> Boiss. et Spruner	2	-	2	-	-
<i>Fritillaria graeca</i> Boiss. et Spruner ssp. <i>thessala</i> (Boiss.) Rix	3	-	1	2	-
<i>Fritillaria imperialis</i> L.	2	-	2	-	-
<i>Fritillaria kotschyana</i> Herb.	1	-	1	-	-
<i>Fritillaria lusitanica</i> Wikstr.	1	-	1	-	-
<i>Fritillaria meleagris</i> L.	110	71	37	2	-
<i>Fritillaria meleagris</i> ssp. <i>burnatii</i> (Planch.) Rix	1	-	1	-	-
<i>Fritillaria messanensis</i> Raf.	5	-	5	-	-
<i>Fritillaria messanensis</i> Raf. ssp. <i>gracilis</i> (Ebel) Rix	6	-	6	-	-
<i>Fritillaria messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman	63	33	5	21	3
<i>Fritillaria montana</i> Hoppe ex W.D.J.Koch	59	-	24	37	-
<i>Fritillaria oranensis</i> Pomel	1	-	1	-	-
<i>Fritillaria orientalis</i> Adams	4	-	4	-	-
<i>Fritillaria pinardii</i> Boiss.	1	-	1	-	-
<i>Fritillaria pudica</i> (Pursh) Spreng.	1	-	1	-	-

Taxa	Total	ZAHO	ZA	CNHM	ZAGR
<i>Fritillaria pyrenaica</i> L.	6	-	6	-	-
<i>Fritillaria ruthenica</i> Wikstr.	3	-	3	-	-
<i>Fritillaria sibthorpiana</i> (Sm.) Baker	1	-	1	-	-
<i>Fritillaria tubiformis</i> Gren. et Godr.	3	-	3	-	-
<i>Fritillaria</i> sp.	10	10	-	-	-
Grand total:	289	114	110	62	3

Herbarium specimens originate from 17 European countries and from Turkey, Algeria and USA (Fig. 6, Fig. 7). The vast majority of herbarium sheets were collected in Croatia (72%). Regarding the Croatian territory, the collecting sites of taxa from ZA are scattered throughout the country, while the localities from ZAHO and CNHM are localized mostly on Velebit Mt, which reflects field activities of the main collectors of those two collections (Ivo Horvat and Ivo Trinajstić, respectively) (Fig. 8).

In the ZA *Herbarium generale* several interesting specimens were discovered. This part of the collection consists of taxa generally not native in Croatia and was formed via exchange with mostly European botanists and herbaria between 1860s and 1930s. During the systematization of these specimens the comparison with available digitalized specimens from other major European herbaria via virtual herbaria (WU, W, JE, B and P) revealed the existence of three probable type specimens. The specimen of *Fritillaria bithynica* Baker present in ZA has the same collector and collection date (Sintenis P.E.E and 1883-04, respectively) and therefore belongs to the same collection as three

specimens kept in JE (JE00008722, JE00008723, JE00008724) and provisionally designated as *Typus* probabiler for *Fritillaria schliemannii* Sint. ex Rodigas published in Ill. Hort. 31: 106 (1884), which is regarded as a synonym of *Fritillaria bithynica* Baker. The specimen of *Fritillaria ehrhartii* Boiss. et Orph. has the label identical to the three specimens stored in JE and WU (JE 00008720, WU0079067, WU0065169) and provisionally designated as *Syntypus* of *Fritillaria ehrhartii* Boiss. et Orph. published in Diagn. pl. orient. sér. 23 (4): 105 (1859). The specimen of *Fritillaria pinardii* Boiss. also belongs to Iter orientale 3931 of collector Sintenis, P.E.E. and belongs to the same collection which was used to describe the taxon *Fritillaria alpina* Freyn et Sint. [protologue by Freyn, 1894: "Paphlagoniae: Tossia in monte Giaurdagh, in pratis alpinis die 17. majo 1892 leg. Sintenis! (Exsicc. no. 3931)"] and therefore the specimen in ZA can also be referred to as *Isotypus* for *Fritillaria alpina* Freyn et Sint. just like the specimens kept in B, JE, KFTA, P and WU (B 10 0279976, JE00009937, JE 00006319, KFTA0002200, MNHN-P-P00730841, MNHN-P-P00730842, WU0065170).

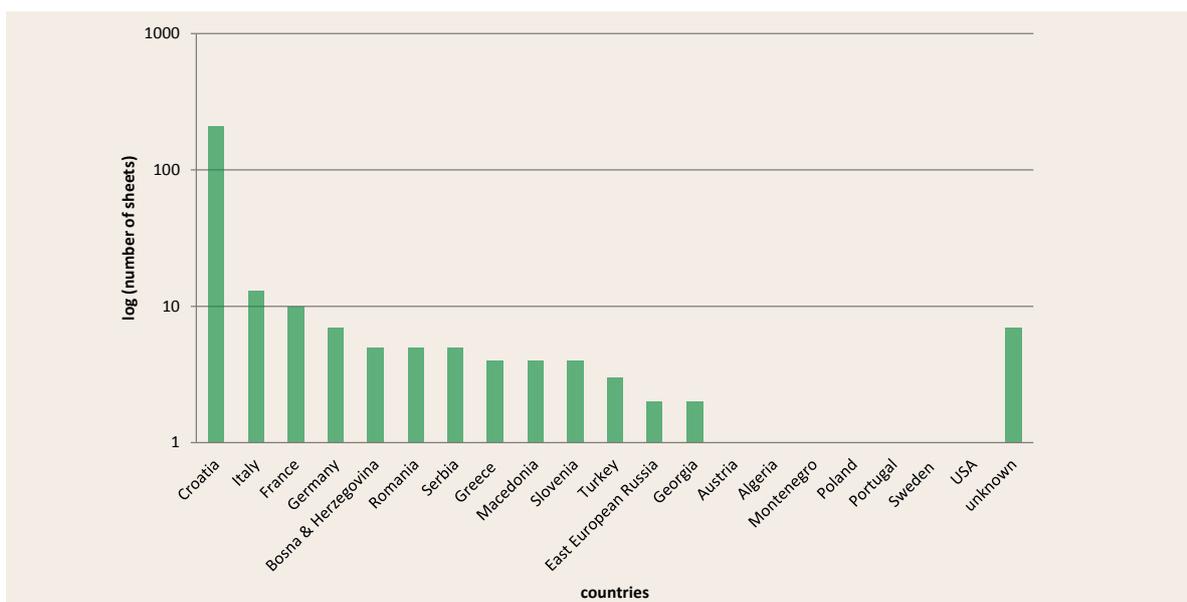


Figure 6. Geographical origin of the *Fritillaria* herbarium sheets stored across studied collections.

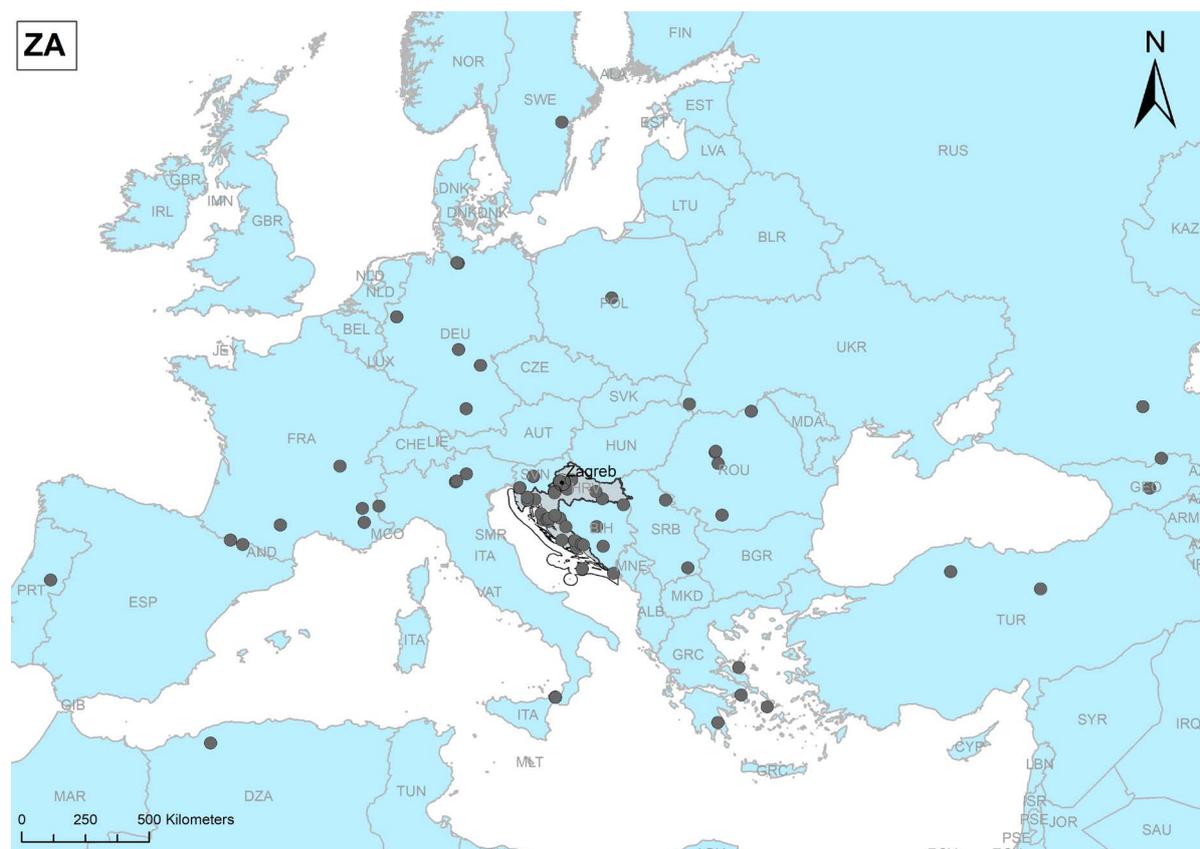


Figure 7. Distribution map of *Fritillaria* herbarium sheets from ZA collection. The record from USA is not shown on the map.

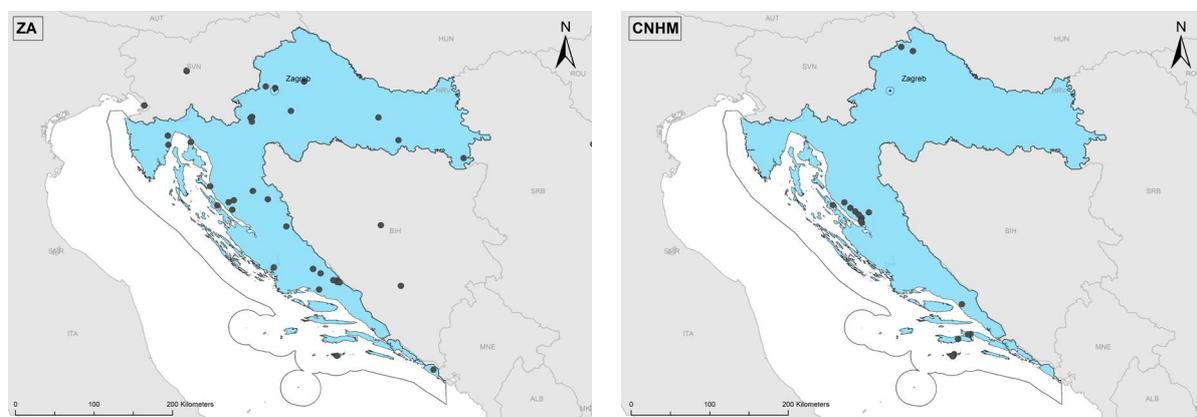


Figure 8a. Distribution maps of *Fritillaria* herbarium sheets from Croatia and neighbouring countries within each studied collection (ZA - Herbarium Croaticum, CNHM - Herbarium of Croatian Natural History Museum).

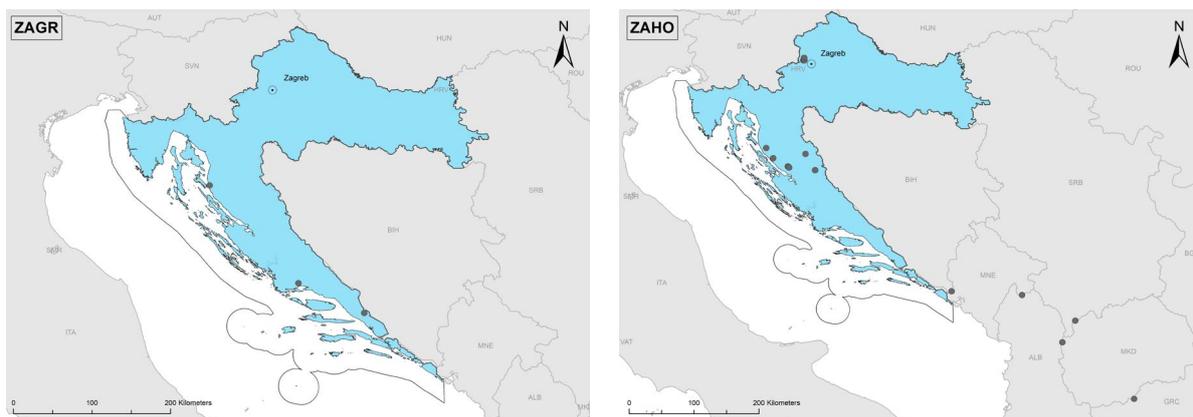


Figure 8b. Distribution maps of *Fritillaria* herbarium sheets from Croatia and neighbouring countries within each studied collection (ZAGR - Herbarium of the Faculty of Agriculture, University of Zagreb, ZAHO - Herbarium of Ivo and Marija Horvat).

The distribution based on herbarium data showed large overlaps with the field record data made by Kranjčev & Šešok (2016), with an exception of *F. messanensis* ssp. *gracilis*. Beside a limited area of distribution in Southern Dalmatia, known prior to our revision, several new, remote localities were recorded (four in Lika and Quarnero, and two in Bosnia & Herzegovina) (Fig. 9). Interestingly, this taxon has been recently recorded in Campania (Southern Italy), too (Peruzzi et al. 2017).

The most productive collector of the studied herbarium material was Ivo Horvat (113 herbarium sheets), who is the lone collector of the ZAHO collection. However, his collection is rather poor in terms of number of taxa and localities. Namely, Horvat collected only two taxa, *F. meleagris* and *F. messanensis* ssp. *neglecta*. He regularly made large

collections from the same locality (e.g. Southern Velebit, NW Croatia, Lika). The largest collection of *F. meleagris* counts as much as 71 herbarium sheets holding 216 specimens, but lacking any locality description, except Croatia. Ivo Trinajstić stored 53 herbarium sheets, mostly in CNHM, Ljudevit Rossi eight, while other 66 collectors contributed with less than seven herbarium sheets each. As much as 17 sheets, exclusively from ZA, are lacking the information about the collector(s) or the collector's name is illegible.

In terms of collecting period, the majority of specimens were collected between 1920s and 1960s (Fig. 10), consisting mostly of the Ivo Horvat's fieldwork. As much as 86 herbarium specimens lack data on collecting period. The average age of the collected specimens is approximately 85 years.

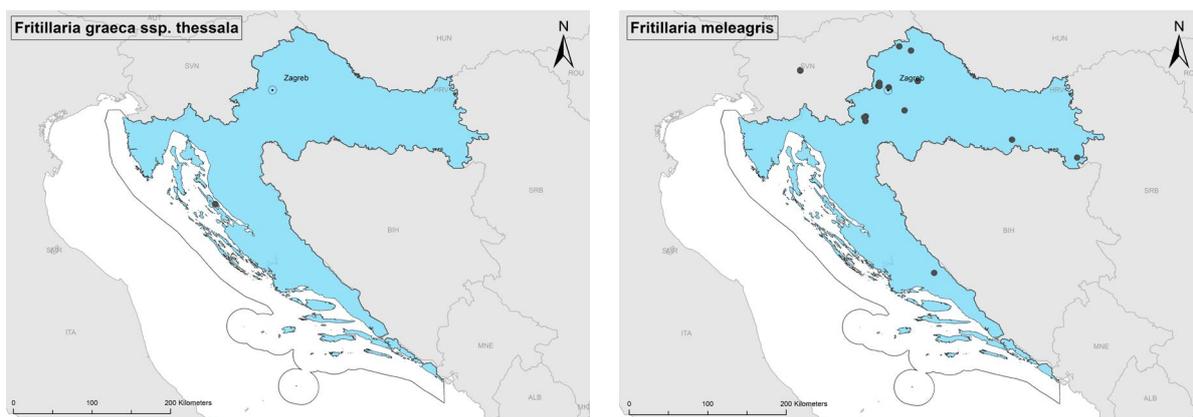


Figure 9a. Individual distribution maps for *Fritillaria* taxa native to Croatia stored across studied collections.

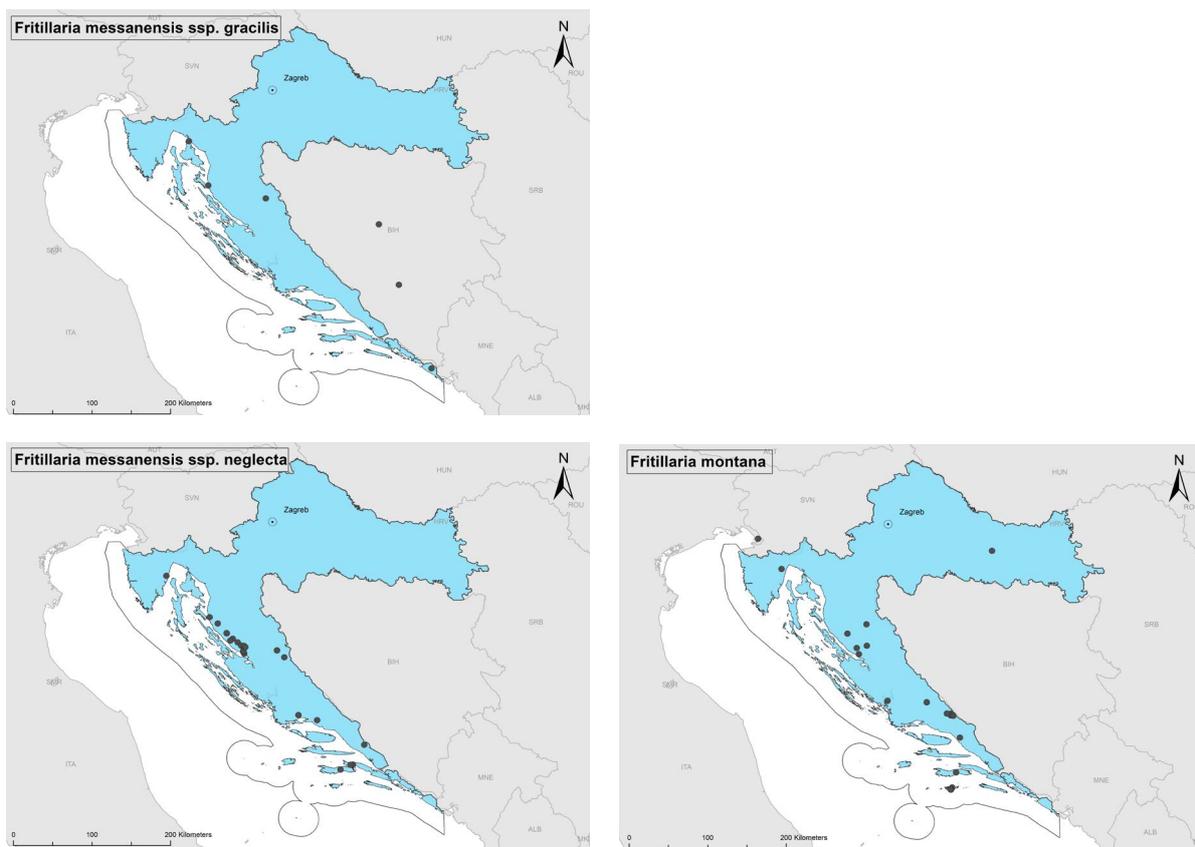


Figure 9b. Individual distribution maps for *Fritillaria* taxa native to Croatia stored across studied collections.

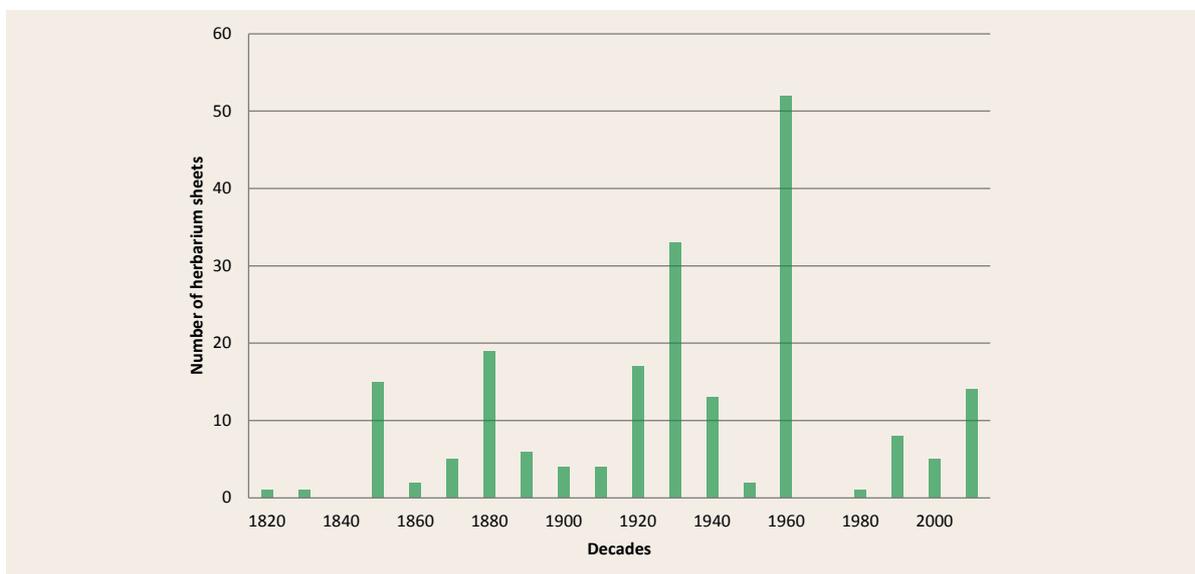


Figure 10. Temporal distribution of the herbarium specimens based on collection period shown in decadal scale.

As expected, 90% of the specimens were collected in flowering season (Fig. 11), when the plants are noticeable in the nature, while the

rest were found in fruit, thus being unable for identification to species rank.

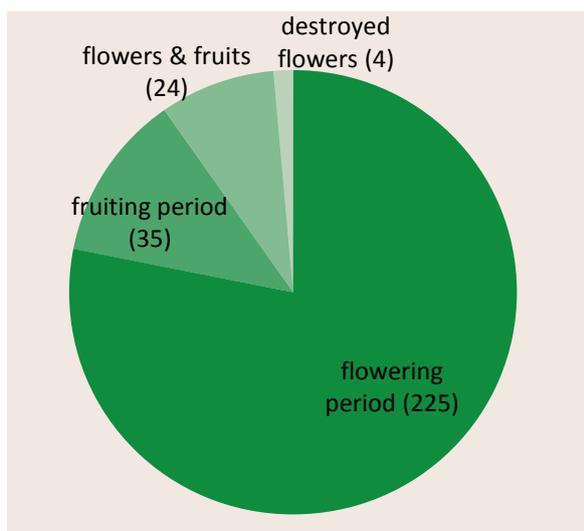


Figure 11. Phenophases of the collected specimens.

In addition, 113 photographs stored in Flora Croatica Database Gallery were carefully checked and revised (Tab. 2), including those that were not designated as public. The majority of revision included *F. messanensis* group and *F. montana*. Several pictures taken on Southern Velebit might lead us to *F. graeca* ssp. *thessala*, a taxa recorded only on the island of Pag (Kranjčev & Šešok 2016). However, visiting the locality in Southern Velebit by the second author in April 2017 has not resulted in finding of *F. graeca* ssp. *thessala*, but only *F. messanensis* ssp. *neglecta* and *F. montana*. Therefore, this photographic record needs further check.

Table 2. The *Fritillaria* taxa revised within Flora Croatica Database Gallery.

ID	Original taxa	Author	Revised taxa
113211	<i>F. messanensis</i> Raf.	Ivica Ljubičić	<i>F. montana</i> Hoppe ex W.D.J.Koch
8433	<i>F. messanensis</i> Raf.	Darko Mihelj	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
8434	<i>F. messanensis</i> Raf.	Darko Mihelj	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
8158	<i>F. messanensis</i> Raf.	Čedomir Šilić	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
8159	<i>F. messanensis</i> Raf.	Čedomir Šilić	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
9585	<i>F. messanensis</i> Raf. ssp. <i>messanensis</i>	Jasenka Topić	<i>F. montana</i> Hoppe ex W.D.J.Koch
9586	<i>F. messanensis</i> Raf. ssp. <i>messanensis</i>	Jasenka Topić	<i>F. montana</i> Hoppe ex W.D.J.Koch
3710	<i>F. messanensis</i> Raf. ssp. <i>gracilis</i> (Ebel) Rix	Gordan Lukač	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
12039	<i>F. montana</i> Hoppe ex W.D.J.Koch	Antun Alegro	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
12040	<i>F. montana</i> Hoppe ex W.D.J.Koch	Antun Alegro	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
12041	<i>F. montana</i> Hoppe ex W.D.J.Koch	Antun Alegro	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
12042	<i>F. montana</i> Hoppe ex W.D.J.Koch	Antun Alegro	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
12043	<i>F. montana</i> Hoppe ex W.D.J.Koch	Antun Alegro	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
11671	<i>F. montana</i> Hoppe ex W.D.J.Koch	Dubravko Šincek	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
72689	<i>F. montana</i> Hoppe ex W.D.J.Koch	Miroslav Marić	<i>F. messanensis</i> Raf. ssp. <i>neglecta</i> (Parl.) Nyman
43049	<i>F. montana</i> Hoppe ex W.D.J.Koch	Dubravko Šincek	? <i>F. graeca</i> Boiss. et Spruner ssp. <i>thessala</i> (Boiss.) Rix
116712	<i>F. montana</i> Hoppe ex W.D.J.Koch	Dubravko Šincek	? <i>F. graeca</i> Boiss. et Spruner ssp. <i>thessala</i> (Boiss.) Rix
116713	<i>F. montana</i> Hoppe ex W.D.J.Koch	Dubravko Šincek	? <i>F. graeca</i> Boiss. et Spruner ssp. <i>thessala</i> (Boiss.) Rix

Conclusion

The fruitful cooperation among the curators of four major Croatian herbaria collections through revision and digitization of *Fritillaria* specimens

resulted in a model useful for future processing of other taxonomically difficult plant groups.

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