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ORCHID (ORCHIDACEAE) DIVERSITY WITHIN *HERBARIUM CROATICUM* (ZA) COLLECTION *SENSU STRICTO*

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Šegota, V. Vilović, T. & Zovko, I.: Orchid (Orchidaceae) diversity within *Herbarium Croaticum* (ZA) collection *sensu stricto*. Nat. Croat., Vol. 28, No. 2, 325-343, 2019, Zagreb.

The collection of orchids within Herbarium Croaticum *sensu stricto* keeps as many as 1373 herbarium sheets, belonging to 91 taxa (25 genera, 76 species, 11 subspecies and four hybrids). The majority of the collection originates from Croatia while the rest are from neighbouring countries (Slovenia, Serbia, Bosnia & Herzegovina, Italy, North Macedonia and Montenegro). The most numerous genera are *Orchis*, *Ophrys* and *Anacamptis*, while the most numerous taxa are *Gymnadenia conopsea*, *Platanthera bifolia*, *Anacamptis morio*, *Neotinea tridentata*, *Cephalanthera longifolia* and *Dactylorhiza maculata*. The oldest herbarium sheets date back to 1883 and the average age of the collection is 113 years. The main collectors are Ljudevit Rossi, Dragutin Hirc and Ambroz Haračić. The collection keeps holotypes and isotypes of *Epipactis rivularis* and *Ophrys dinarica*, while exsiccates of *Malaxis monophyllos*, *Herminium monorchis* and *Ophrys* × *muellneri* are the only evidence of their existence in Croatia. All sheets were digitized and are publically available via virtual herbarium.

Key Words: Croatia, digitization, herbarium, orchids

Šegota, V. Vilović, T. & Zovko, I.: Raznolikost orhideja (Orchidaceae) u zbirci Hrvatskog Herbarija *sensu stricto* (ZA). Nat. Croat., Vol. 28, No. 2., 325-343, 2019, Zagreb.

Zbirka orhideja u Hrvatskom herbariju *sensu stricto* sadrži ukupno 1373 herbarijskih listova, koji pripadaju 91 svojti (25 rodova, 76 vrsta, 11 podvrsta i četiri hibrida). Većina zbirke potječe iz Hrvatske i nekoliko susjednih zemalja (Slovenija, Srbija, Bosna i Herzegovina, Italija, Sjeverna Makedonija i Crna Gora). Najbrojniji rodovi su *Orchis*, *Ophrys* i *Anacamptis*, a vrste *Gymnadenia conopsea*, *Platanthera bifolia*, *Anacamptis morio*, *Neotinea tridentata*, *Cephalanthera longifolia* i *Dactylorhiza maculata*. Najstariji primjerci potječu iz 1883., a prosječna starost zbirke je čak 113 godina. Glavni sakupljači su Ljudevit Rossi, Dragutin Hirc i Ambroz Haračić. U zbirci se čuvaju holotipovi i izotipovi vrsta *Epipactis rivularis* i *Ophrys dinarica*, a eksikati *Malaxis monophyllos*, *Herminium monorchis* i *Ophrys* × *muellneri* su jedini dokaz postojanja ovih vrsta u Hrvatskoj. Svi herbarijski primjerci su digitalizirani i javno su dostupni preko virtualnog herbarija.

Ključne riječi: Hrvatska, digitalizacija, herbarij, orhideje

INTRODUCTION

The orchid family seems to represent the most fascinating plant family for professional and amateur botanists worldwide. The first interest for orchids in Croatia can be traced back to the beginning of the 20th century, when orchids of island Lošinj (Fleischmann, 1904) and Dalmatia (Hayek, 1913) were studied. In the following period, the focus of orchidological fieldwork in Croatia mostly remained on the Adriatic coast (Hellmayr, 1939-1941; Löschl, 1971; Vöth

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& Löschl, 1978; Göltz & Reinhard, 1986). During the last 20 years, several noteworthy contributions to the Croatian orchid flora were published (Perko, 1998; Šegota & Alegro, 2011; Šegota *et al.*, 2012; Vuković *et al.*, 2013; Zadravec *et al.*, 2013; Jeričević *et al.*, 2015; Zadravec *et al.*, 2015; Borovečki-Voska, 2016). Moreover, several important field inventories of particular areas were performed throughout Croatia, e.g. Žumberak Mt. (Vrbek & Fiedler, 1998), Medvednica Mt. (Hršak *et al.*, 1999; Zadravec *et al.*, 2014), island of Vis (Kranjčev, 2001; Broggi & Burri, 2006), Istria and Quarnerro Islands (Kerschbaumsteiner *et al.*, 2002; Hertel & Hertel, 2003; Griebel, 2009; Vuković *et al.*, 2011), Požega Valley (Zima *et al.*, 2006) and Krka National park (Vuković *et al.*, 2017). Additionally, several popular orchid guidebooks have been published recently (Crvenka, 1999; Pavlus, 2004; Kranjčev, 2005; Borovečki-Voska, 2010). According to Flora Croatica Database, there are 183 taxa within 27 genera of orchids in Croatia, 19 of which are endemic (Nikolić, 2019).

The Division of Botany of the Faculty of Science in Zagreb holds two officially registered herbarium collection – *Herbarium Croaticum* (ZA) and *Herbarij Ive i Marije Horvat* (ZAHO) (Thiers, 2019). The ZA collection was founded in 1880 and includes herbarium sheets collected by various researchers during the past 200 years. With over 200.000 herbarium sheets it is the oldest and the largest herbarium collection in Croatia. It holds three separate units, namely Herbarium Generale (historical collection formed via exchange with other world herbaria), Herbarium Croaticum *sensu stricto* (collection of flora of Croatia and, partially, of adjacent countries) and Herbarium Cryptogamicum (collection of algae, mushrooms, lichens and bryophytes).

Besides for the taxonomy and systematics as their primary purpose, herbarium collections can be also used for the analysis of wide range of environmental topics, depending on types of data that can be obtained from specimens. Containing the information on meta-data (date and location), phenotype, phenology, molecular data and biotic interactions of collected herbarium specimens, in recent years few new approaches emerged, such as studies on invasions, habitat change, climate change and pollution (Lang *et al.*, 2019). For example, using herbarium data, a phenological response to climate change in terrestrial orchids has been proven in Hungary, demonstrating that the majority of orchids have shifted their yearly mean flowering to earlier dates during the past 50 years (Molnár *et al.*, 2012a).

Today's information technologies are greatly accelerating scientific research, by providing easy and flexible ways of accessing data collected from all around the world, including herbarium collections (Kislov *et al.*, 2017). At present, the formation of Internet resources of herbarium collections is an important branch of herbarium management, and digitization of collections is a recent trend in many herbaria (Flannery, 2012). Public access to such information via herbarium-based databases (virtual herbaria) makes collections more broadly useful and improves scientific research (Smith & Blagoderov, 2012; Penn *et al.*, 2017). Bearing in mind that more than a half of herbarium collections from some regions of the world may be incorrectly identified (Goodwin *et al.*, 2015), digital collections permit remote access by specialists and more precise identifications.

World leaders in herbarium digitization are Museum of Natural History in Paris (P) with 5.3 million digitized specimens, Naturalist in Leiden, Netherlands (L) with 4 million digitized specimens, Institute of Botany, New York Botanical Garden (NY) with 2 million digitized specimens and China Academy of Sciences (PE) with 1.8 million digitized specimens (Seregin, 2016). Moreover, NY is currently adding 20.000 scans per month, while Smithsonian Institution in Washington (US) is adding 4.000 scans per day, and Royal Botanic Gardens in Kew (K) and The Natural History Museum in London (BM) are adding 3.000 scans per day (Seregin, 2016). However, at present, over one million of specimens is kept in at least 61 herbaria (Seregin, 2016), thus the majority of world herbaria remains inaccessible via Internet.

In last two decades, several studies based on Croatian herbarium collections have been published (Vidović, 2001; Hršak, 2002; Ževrnja & Vladović, 2008; Strgulc Krajšek *et al.*, 2009; Šegota *et al.*, 2017; Britvec *et al.*, 2018). As for orchids, recent analyses of herbarium collection were made for all Hungarian (Molnár *et al.*, 2012b) and partially Serbian (Djordjević *et al.*, 2017) herbaria.

Digitization of herbarium sheets within ZA collection has initiated in 2015, after the purchase, extensive testing and technical improvements of the equipment (Šegota *et al.*, 2017). As many as 28.251 herbarium sheets from ZA and 4.352 herbarium sheets from ZAHO have been digitized so far, out of which 8.322 sheets from ZA and 1.207 from ZAHO have been scanned and are accessible via ZA & ZAHO Virtual Herbarium (Rešetnik & Šegota, 2019) and Flora Croatica Database (Nikolić, 2019).

The aims of this research were to (a) analyse the diversity of the orchid family within Herbarium Croaticum *sensu stricto* collection, (b) perform revision of some genera and taxa, and c) estimate the average time for digitization and detect the bottlenecks within this process.

MATERIAL AND METHODS

For the purpose of this survey, we studied in 2016 and 2017 the orchids within Herbarium Croaticum *sensu stricto* collection (in the following text as ZA), meaning that the specimens from world collection (Herbarium Generale) were not included, in order to study solely the distribution of orchids in Croatia and adjacent countries. Revision of the most of the herbarium material was not done, since majority of orchid taxa are generally difficult to be identified in dry and colourless state. In case of typical subspecies (e.g. *Ophrys sphegodes*, O. *sphegodes* subsp. *sphegodes*, *Anacamptis laxiflora*, *A. laxiflora* subsp. *laxiflora*), we left those taxa separately as noted in collection, since we were unable to clarify which subspecies specimens labelled on species level actually belong to. As for the taxa with problematic geographical distribution (e.g. *Neotinea tridentata* subsp. *commutata* and *Anacamptis morio* subsp. *picta*), we failed to resolve their identity, thus we left their original nomenclature. In contrast, the specimens named as *Nigritella nigra* were successfully identified as *Gymnadenia rhellicani,* based on distinct serrations along the margin of lower bracts (Delforge, 2006). Revision of the genus *Himantoglossum* was successfully performed. In addition, the whole

collection is publically available via Flora Croatica Database, as well as ZA & ZAHO Virtual Herbarium, for further experts' examinations.

The pre-scanning process included cleaning, dusting and translocation of plant material to new format of herbarium sheet adequate for scanning, mounting with pH neutral adhesive tape and gluing of herbarium labels (Šegota *et al*., 2017). If the label was covered by a plant or folded, we made it clearly visible, while important broken parts of plant were assembled back to the specimens via adhesive tape or stored within a special envelope. Additionally, a ZA stamp, standard colour palette and scale were provided on each herbarium sheet before the scanning. The metadata of the digital herbarium were stored in Flora Croatica Database (Nikolić, 2019), and included all information presented on the herbarium labels; namely, the ID of herbarium sheet, taxon name, geographical reference, habitat data, collector and identifier of each specimen, dates of collection and collection number. Prior to digitizing of the collection, we verified the taxonomy and revealed synonyms.

We used inversed Epson Expression 11000XL Pro A3 scanner to make image files with .tiff extension, named by herbarium sheet's ID. There are no specific regulations regarding image resolution in herbaria worldwide, thus we decided to follow recommendations from Seregin (2016), and use a resolution of 300 dpi for regular herbarium sheets and 600 dpi for those of highest importance – exemplary specimens or type specimens. The scanned images in original resolution and extension are kept offline as four backups. Finally, high resolution images were uploaded and published in Flora Croatica Database, as well as in ZA & ZAHO Virtual Herbarium. Based on the experience gained through orchid digitization, we estimated the average time necessary for digitizing one herbarium sheet.

Taxa names (except type specimens) are cited in text without the author names for simplicity, while the full names are listed in Appendix 1. The nomenclature used in this paper follows phylogenetic approach of Bateman *et al.* (1997) applied in World Checklist of Kew Gardens (WCSP, 2019) and local floras (e.g. Djordjević *et al*., 2018). However, we left in brackets the names still in use in Flora Croatica Database.

RESULTS AND DISCUSSION

As many as 1373 herbarium sheets with orchids were restored and digitized, belonging to 25 genera, 91 taxa, including 11 subspecies and four hybrids. Several specimens remained unidentified, namely 24 herbarium sheets of the genus *Epipactis*, and four sheets of the genus *Ophrys*. Similarly, the majority of the unidentifiable orchid records from Herbarium Database of Hungarian Orchids belong to the taxonomically critical genus *Epipactis* (Molnár, 2012b). This is not unusual, since orchids are being identified using, in most cases, colour and shape of various floral structures, generally not recognisable on dried plants. In Croatia, 183 orchid taxa have been recorded so far (Nikolić, 2018), thus ZA collection encompasses 50 % of the national diversity of orchids. The full list of orchid taxa from ZA is provided in Appendix 1. Regarding the Red listed taxa (Nikolić & Topić, 2005), all of them are represented within ZA collection, except *Orchis spitzelii* Saut. ex Koch. Two holotypes have been found, namely, *Epipactis rivularis* Kranjčev et Čičmir (ZA15691) (Fig. 1) and *Ophrys dinarica* Kranjčev et P. Delforge (ZA14344) (Fig. 2), as well as isotypes of both taxa (ZA28360, ZA14344) (Fig. 2).

Fig. 1. Holotype of *Epipactis rivularis* Kranjčev et Čičmir (ZA15691)

Fig. 2. Holotype and isotype of *Ophrys dinarica* Kranjčev et P. Delforge (ZA14344)

The most numerous orchid genera within ZA in terms of number of taxa are *Ophrys* (18), *Orchis* (11), *Anacamptis* (9) and *Epipactis* (8). As for the number of herbarium sheets, the most numerous genera are *Orchis* (213), *Ophrys* (181), *Anacamptis* (150), *Cephalanthera* (118), *Dactylorhiza* (117), *Epipactis* and *Gymnadenia* (109 each) (Fig. 3).

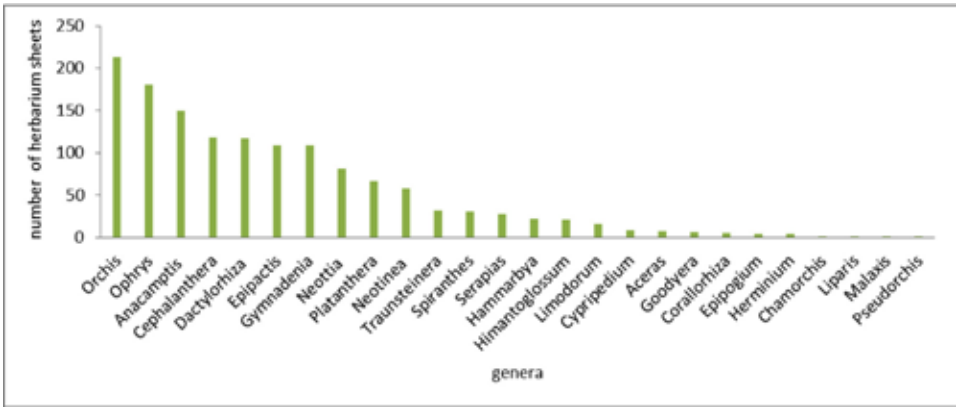

Fig. 3. The most numerous orchid genera in terms of number of herbarium sheets

As for the number of herbarium sheets of particular taxa, the most numerous orchids are *Gymnadenia conopsea* (78), *Platanthera bifolia* (59), *Anacamptis morio* (52), *Neotinea tridentata* (50), *Cephalanthera longifolia* (47) and *Dactylorhiza maculata* (46) (Fig. 4). On the other hand, 40 % of taxa are represented with less than five herbarium sheets, including 19 taxa with only one herbarium sheet.

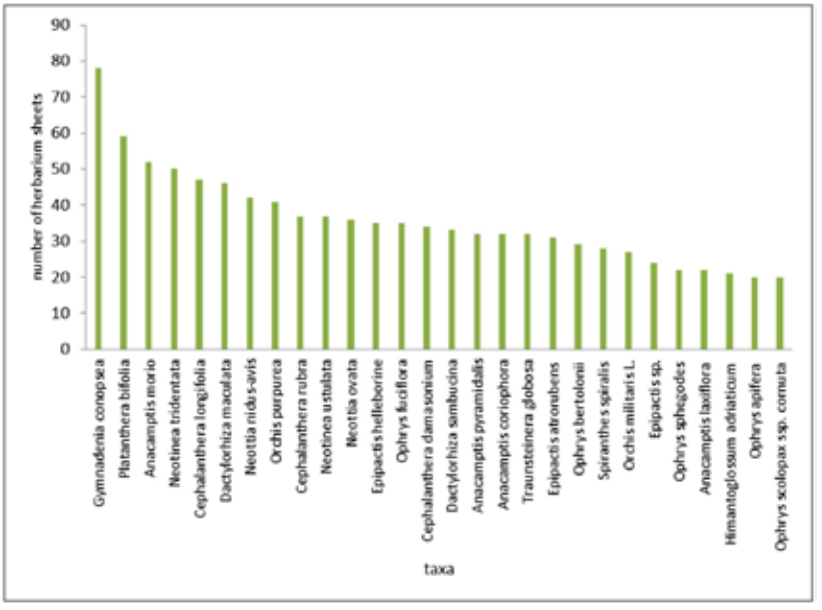

Fig. 4. The most numerous orchid taxa within ZA collection

As expected, the majority of herbarium sheets (90 %) had been collected in Croatia, while the rest originate mainly from the neighbouring countries Slovenia (6.7 %), Serbia (1.2 %), Bosnia & Herzegovina (0.9 %), Italy (0.9 %), while a single herbarium sheet was collected from both North Macedonia and Montenegro (Fig. 5).

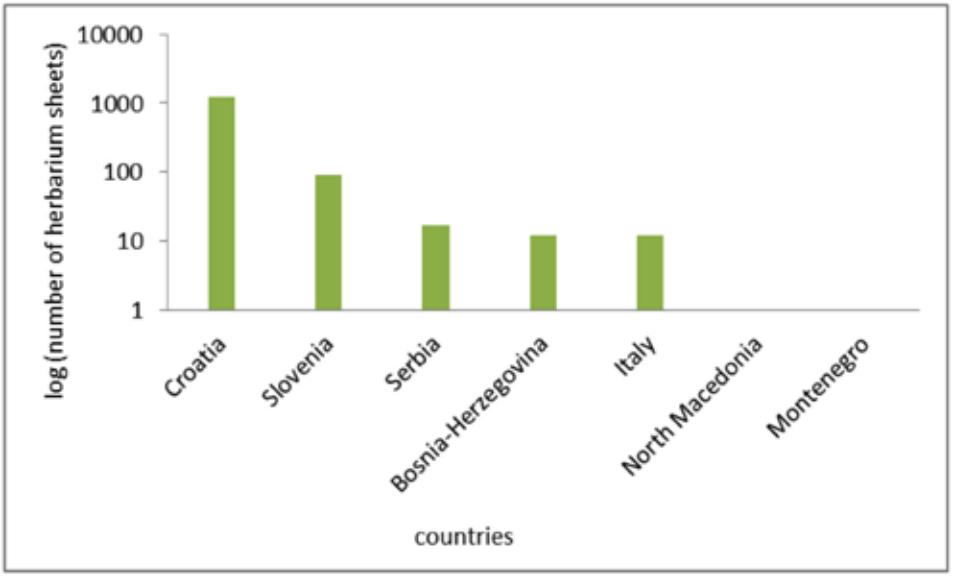

Fig. 5. Geographical origin of orchids stored within ZA collection

Several rare taxa, with no records from Croatia, were collected only in Slovenia, namely *Chamorchis alpina*, *Gymnadenia miniata*, *Gymnadenia* × *heufleri*, *Hammarbya paludosa*, *Liparis loeselii, Neotinea* × *dietrichiana* and *Pseudorchis albida*. Interestingly, we have found a few sheets with no other proof of their occurrence in Croatia, apart from the herbarium sheets, and these were *Malaxis monophyllos* from Ivanščica Mt. (Fig. 6), *Herminium monorchis* from Čabar (Fig. 7) and *Ophrys* × *muellneri* from the island of Lošinj (Fig. 8). Similarly, the finding of the same taxa (*Malaxis monophyllos* and *Herminium monorchi*) in Hungarian herbaria validated the unambiguous presence of these two extinct Hungarian species (Molnár *et al.*, 2012b). In addition, herbarium sheets of *Ophrys fusca* ssp. *iricolor*, *Ophrys tenthredinifera* and *Epipactis rivularis* found in ZA represent a valuable confirmation of these rare species in Croatia.

Regarding the geographical references, 10 % of sheets had no geodata on the herbarium label, or it was not possible to decode the collectors' handwriting nor to find historical toponyms on the available maps. The available records were used to produce a distributional map, shown in Fig. 9. Regarding the time of

collection, 17.6 % of herbarium sheets lacks temporal information. However, we can aproximate the timeframe for majority of records, due to the known life span of collectors. The oldest sheets are *Orchis pallens* (ZA6818) and *Epipactis atrorubens* (ZA42165), dating back to 1833, both collected by Ljudevit Vukotinović in March 1833 in Šestine near Zagreb (*kod Šestinah*) and on 13th and 26th July on Plešivica and Oštrc in Samoborsko gorje Mts. (*na Plišivici i Oštrcu u Gori Samoborskoj*), respectively. In comparison, in Hungarian orchids collection 10 % of herbarium sheets lack the exact date of collection, while the oldest sheet dates back to 1804 (Molnár *et al*., 2012b). Among rare and threatened orchid taxa in the Central Balkans, the oldest specimen, *Epipactis palustri*s, was collected as early as in 1851 (Djordjević *et al*., 2017). As many as 67 % of orchid herbarium sheets in ZA had been collected in the period from 1870 to 1910, while in the last 100 years, only 21.5 % of sheets were stored in ZA (Fig. 10). Solely 14 sheets were collected and stored in ZA in the 21st century. Strikingly, the average age of the collected sheets is 113 years. Evidently, the majority of the orchid collection has been formed during the end of 19th and the beginning of 20th century, revealing almost complete lack of collection effort during the last century. This is at least partially due to the legal restrictions, since the whole orchid family has been strictly protected over the last 50 years, and the prohibition of collection has been in force. On the other hand, along with rapid development of colour photography, it became easier to identify species using photographs, rather than using dried specimens normally exhibiting severe colour change, therefore the "need" for collection is far less justified in modern times. In Hungary, similar decrease in the number of collected orchids is coincident with the shift from floristics and taxonomy to other fields of botany, pointing that collection has become somewhat "old-fashioned" (Molnár *et al*., 2012b). On the other hand, the growing importance of legal species protection does not seem to have played a significant role, since there has also been a reduction in collecting other, non-protected taxa (Molnár *et al*., 2012b).

The main collectors were Ljudevit Rossi with 330 herbarium sheets, Dragutin Hirc with 269 sheets and Ambroz Haračić with 128 sheets (Fig. 11), encompassing 53 % of the collection. As many as 94 collectors participated in total, however 83 % of all collectors have collected less than ten sheets each. In Hungarian orchid collection, 36 % of the collectors have only one sheet, whereas two-thirds of the collection comes from 10 % of the collectors (Molnár *et al*., 2012b). In ZA, only 14 sheets lack the information on collectors, or the collector could not be identified through the handwriting.

Fig. 6. Specimen of rare *Malaxis monophyllos* from Ivanščica Mt. (ZA7111)

Fig. 7. Specimens of rare *Herminium monorchis* from Čabar (ZA15692)

Fig. 8. Specimen of rare *Ophrys* × *muellneri* from island of Lošinj (ZA27909)

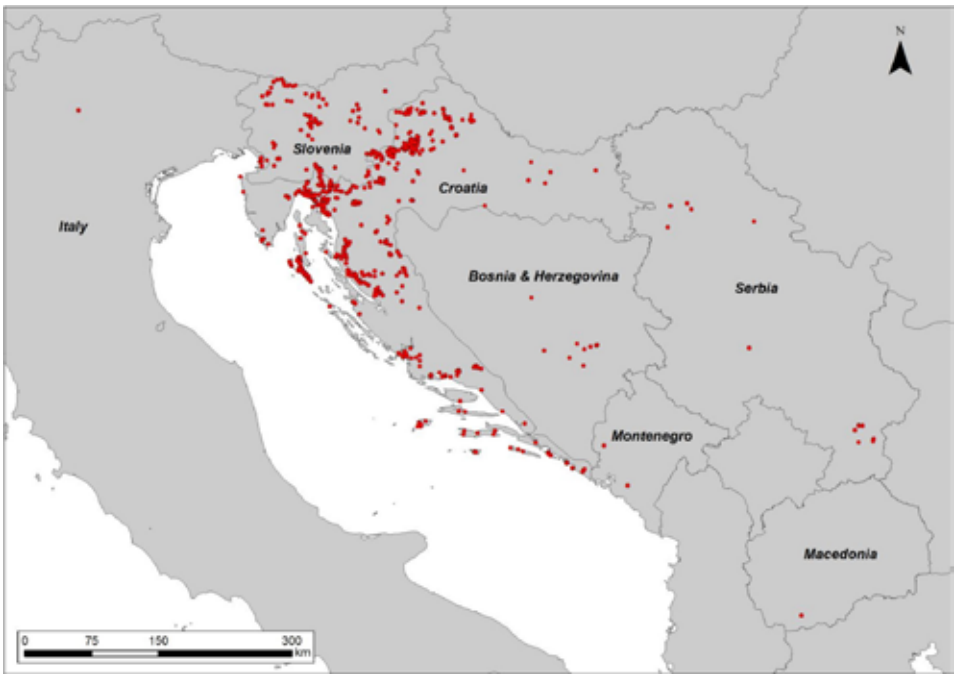

Fig. 9. Distribution map of orchid taxa from ZA collection

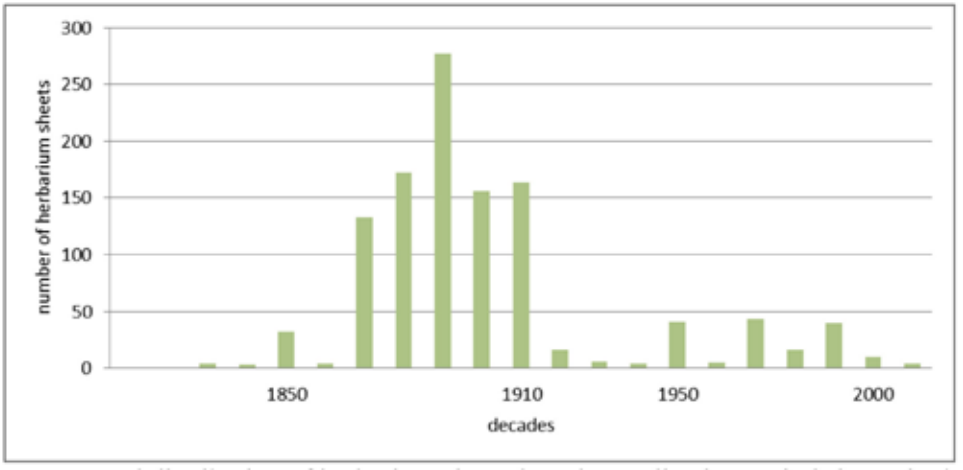

Fig. 10. Temporal distribution of herbarium sheets based on collection period shown in decadal scale

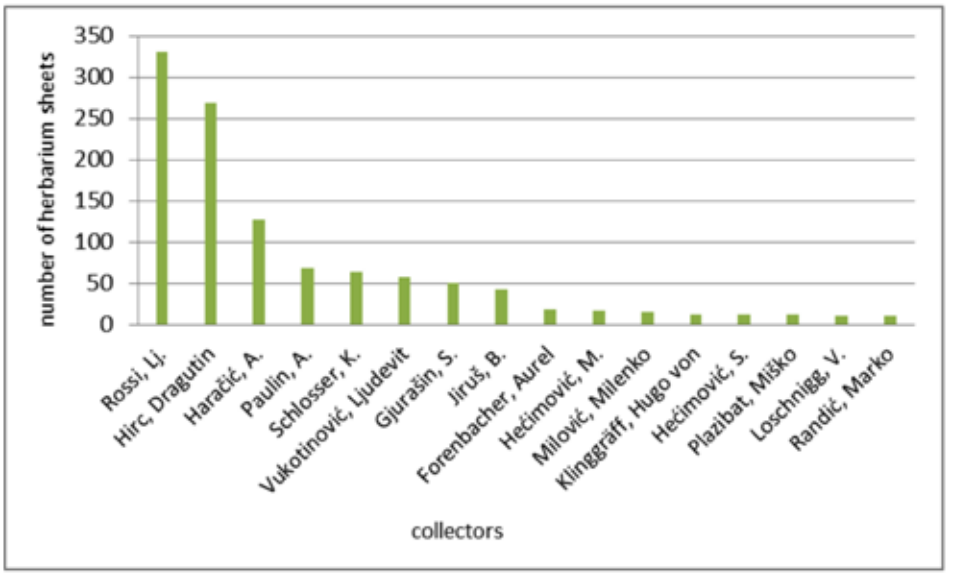

Fig. 11. The most productive orchid collectors in ZA

Finally, based on the experience gained through the entire process, i.e. the specimens' preparation, translocation and restauration, label transcription, georeferencing in GIS environment, scanning, photo processing and uploading to the database, we have managed to estimate the average time of 15 minutes required to fully digitize a single herbarium sheet. It was observed that the label transcription (the decoding of the handwriting) as well as georeferencing (searching for toponyms) represent the main bottlenecks during the digitization process.

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Appendix 1. Taxa list of orchids in ZA collection. The nomenclature follows Bateman *et al.* (1997) and World Checklist of Kew Gardens (WCSP, 2019). Synonyms in brackets are names used in Flora Croatica database.

Anacamptis
Anacamptis coriophora (L.) R.M.Bateman, Pridgeon & M.W.Chase
(syn. *Orchis coriophora* L., incl. *Orchis coriophora* L. ssp. *fragrans* (Pollini) K.Richt.)
Anacamptis laxiflora (Lam.) R.M.Bateman, Pridgeon & M.W.Chase
(syn. *Orchis laxiflora* Lam.)
Anacamptis laxiflora (Lam.) R.M.Bateman, Pridgeon & M.W.Chase ssp. *laxiflora*
(syn. *Orchis laxiflora* Lam. ssp. *laxiflora*)
Anacamptis morio (L.) R.M.Bateman, Pridgeon & M.W.Chase
(syn. *Orchis morio* L.)
Anacamptis morio subsp. *picta* (Loisel.) Jacquet & Scappat.
(syn. *Orchis morio* L. ssp. *picta* (Loisel.) K.Richt.)
Anacamptis palustris (Jacq.) R.M.Bateman, Pridgeon & M.W.Chase subsp. *palustris*
(syn. *Orchis laxiflora* Lam. ssp. *palustris* (Jacq.) Bonnieret Layens)
Anacamptis palustris subsp. *elegans* (Heuff.) R.M.Bateman, Pridgeon & M.W.Chase
(syn. *Orchis laxiflora* Lam. ssp. *elegans* (Heuff.) Soó)
Anacamptis papilionacea (L.) R.M.Bateman, Pridgeon & M.W.Chase
(syn. *Orchis papilionacea* L.)
Anacamptis pyramidalis (L.) Rich.
Cephalanthera
Cephalanthera damasonium (Mill.) Druce
Cephalanthera longifolia (L.) Fritsch
Cephalanthera rubra (L.) Rich.
Chamorchis
Chamorchis alpina (L.) Rich.
Corallorhiza
Corallorhiza trifida Châtel.
Cypripedium
Cypripedium calceolus L.
Dactylorhiza
Dactylorhiza cordigera (Fr.) Soó
Dactylorhiza incarnata (L.) Soó
Dactylorhiza maculata (L.) Soó
Dactylorhiza majalis (Rchb.) P.F.Hunt et Summerh.
Dactylorhiza sambucina (L.) Soó
Dactylorhiza viridis (L.) R.M.Bateman, Pridgeon & M.W.Chase
(syn. *Coeloglossum viride* (L.) Hartm.)
Epipactis
Epipactis atrorubens (Hoffm.) Besser
Epipactis helleborine (L.) Crantz
Epipactis microphylla (Ehrh.) Sw.
Epipactis nordeniorum Robatsch
Epipactis palustris (L.) Crantz
Epipactis pontica Taubenheim
(syn. *Epipactis helleborine* (L.) Crantz ssp. *pontica* (Taubenh.) H.Sund.)
Epipactis purpurata Sm.
Epipactis rivularis Kranjčev et Čičmir
Epipogium

Epipogium aphyllum Sw.
Goodyera
Goodyera repens (L.) R.Br.
Gymnadenia
Gymnadenia conopsea (L.) R.Br.
Gymnadenia densiflora A. Dietr.
(syn. *Gymnadenia conopsea* (L.) R.Br. ssp. *densiflora*) (Wahlenb.) K.Richt.
Gymnadenia miniata (Crantz) Hayek
(syn. *Gymnadenia rubra* Wettst.)
Gymnadenia nigra (L.) Rchb.f.
(syn. *Nigritella nigra* (L.) Rchb.)
Gymnadenia odoratissima (L.) Rich.
Gymnadenia × *heufleri* (A.Kern.) Wettst.
Hammarbya
Hammarbya paludosa (L.) Kuntze
Herminium
Herminium monorchis (L.) R.Br.
Himantoglossum
Himantoglossum adriaticum H.Baumann
Limodorum
Limodorum abortivum (L.) Sw.
Liparis
Liparis loeselii (L.) Rich.
Malaxis
Malaxis monophyllos (L.) Sw.
Neotinea
Neotinea lactea (Poir.) R.M.Bateman, Pridgeon & M.W.Chase
(syn. *Orchis lactea* Poir.)
Neotinea maculata (Desf.) Stearn
Neotinea tridentata (Scop.) R.M.Bateman, Pridgeon & M.W.Chase
(= *Orchis tridentata* Scop.)
Neotinea tridentata (Scop.) R.M.Bateman, Pridgeon & M.W.Chase ssp. *commutata* (Tod.) R.M.Bateman, Pridgeon & M.W.Chase
(syn. *Orchis tridentata* Scop. ssp. *commutata* (Tod.) Nyman)
Neotinea ustulata (L.) R.M.Bateman, Pridgeon & M.W.Chase
(= *Orchis ustulata* L.)
Neotinea × *dietrichiana* (Bogenh.) H.Kretzschmar, Eccarius & H.Dietr.
Neottia
Neottia cordata (L.) Rich. (syn. *Listera cordata* (L.) R.Br.)
Neottia nidus-avis (L.) Rich.
Neottia ovata (L.) Bluff & Fingerh. (syn. *Listera ovata* (L.) R.Br.)
Ophrys
Ophrys apifera Huds.
Ophrys bertolonii Moretti
Ophrys bombyliflora Link
Ophrys fuciflora (F.W.Schmidt) Moench
Ophrys fuciflora subsp. *fuciflora* (syn. *Ophrys dinarica* Kranjčev et P.Delforge)
Ophrys fusca Link
Ophrys fusca Link ssp. *iricolor* (Desf.) K.Richt.
Ophrys insectifera L.
Ophrys lutea Cav.

Ophrys scolopax Cav.
Ophrys scolopax Cav. ssp. *cornuta* (Steven) E.G.Camus
Ophrys sphegodes Mill.
Ophrys sphegodes subsp. *araneola* (Rchb.) M.Laínz (= *Ophrys sphegodes* Mill. ssp. *tommasinii* (Vis.) Soó)
Ophrys sphegodes Mill. ssp. *atrata* (Rchb.f.) A.Bolòs
Ophrys sphegodes Mill. ssp. *sphegodes*
Ophrys tenthredinifera Willd.
Ophrys × *albertiana* E.G.Camus
Ophrys × *muellneri* H.Fleischm.
Orchis
Orchis anthropophora (L.) All. (= *Aceras anthropophorum* (L.) W.T.Aiton)
Orchis italica Poir.
Orchis mascula (L.) L.
Orchis mascula (L.) L. subsp. *speciosa* (Mutel) Hegi (= *Orchis mascula* (L.) L. ssp. *signifera* (Vest) Soó)
Orchis militaris L.
Orchis pallens L.
Orchis pauciflora Ten. (= *Orchis provincialis* Balb. ssp. *pauciflora* (Ten.) Camus)
Orchis provincialis Balb. Ex Lam. & DC.
Orchis purpurea Huds.
Orchis quadripunctata Cirillo ex Ten.
Orchis simia Lam.
Platanthera
Platanthera bifolia (L.) Rich.
Platanthera chlorantha (Custer) Rchb.
Pseudorchis
Pseudorchis albida (L.) Á.Löve et D.Löve
Serapias
Serapias cordigera L.
Serapias lingua L.
Serapias parviflora Parl.
Serapias vomeracea (Burm. f.) Briq.
Spiranthes
Spiranthes aestivalis (Poir.) Rich.
Spiranthes spiralis (L.) Chevall.
Traunsteinera
Traunsteinera globosa (L.) Rchb.