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Forensic potential of pollen grains: A case study from continental Croatia (town of Donja Stubica)

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Abstract

Background and Purpose: *Pollen grains can come as "invisible" traces during forensic palynological expertise. As forensic palynology is not carried out in Croatia at all, the main aims of our work were to establish an initial palynological collection for a limited area in Croatia and determine its forensic potential.*

Materials and Methods: *For our case study, we chose a typical town from continental Croatia, Donja Stubica, in which there had been no previous floristic studies. The field survey was conducted during 2005, 2007, and 2021. Floristic and palynological analysis was carried out with an emphasis on the forensic potential of pollen grains.*

Results: *A total of 141 representative plant taxa with pollen samples were collected. The taxonomic analysis showed that the area for the case study was chosen well. Palynological analysis showed that the highest number of taxa had pollen grains that were spheroidal in shape (60%), medium sized (70%), and colporate (41%). The pollen of the majority of taxa had excellent or very important forensic potential, due to their dispersal by insects (60%), a combination of two dispersal methods (23%), and autogamy (1%). In addition, due to the limited distribution of certain rare plants (e.g.,* Iris sibirica *subsp.* sibirica *in Croatia), their pollen has an even greater forensic potential than the similar pollen of related, more widespread taxa.*

Conclusion: *The generated palynological collection, due to the great forensic potential, could be used in the future as a comparative, and at least partly as a reference forensic collection, applicable in specific cases of legal pollen expertise in Croatia.*

INTRODUCTION

Because of their microscopic size (usually less than 100 μm), outer wall (exine) made of sporopollenin, and often greater release than needed by a plant for sperm cell production and transfer, pollen grains are present all around us as well as on/inside us *(1,2)*. By applying methods of forensic palynology, we can use pollen grains as nearly invisible traces that can (dis)affirm a suspected criminal's alibi. In a legal expertise *(3,5)*, there must be two types of samples: undisputed and disputed. Undisputed ones (control, reference) are samples of surfaces that are in contact with air at the scene of a crime or the place where it is suspected that a crime has been committed. In these samples, the total number of pollen grains is determined, and their analysis is carried out, which results in a "fingerprint" of a particular locality. All other (disputed) forensic samples are compared with the samples obtained in this way.

Disputed samples are those whose relevance to a criminal offense must be determined by palynological expertise. The task of the palynologist is to confirm that the pollen grains from the disputed sample are the same as those from the relevant undisputed sample taken from the primary or secondary crime site and exempted if possible, during the primary processing of the crime site. The total number of pollen and spores identified in a sample (so-called "pollen assemblage") provides a "pollen print" for a given location. This pollen print then becomes the "control sample" for a specific location. To identify pollen grain types from forensic samples, it is necessary to collect pollen/plants/plant parts and (photo) document, identify and, if possible, herborize each individual plant taxon (voucher) from the crime site *(4,5)*. This part of the expertise is vital, because any incorrect identification of pollen types from a forensic sample can lead to mistaken interpretations about the very location of the crime scene. Forensic pollen samples can be recovered from almost any surface exposed or in contact with air, such as soil, dirt, dust collected from persons/items, hair, textile clothing/usable items, stomach contents/sinus cavities of corpses, illicit drugs – *Cannabi*s, cocaine, heroin; honey, fruits, tea, coffee, tobacco, furniture, stamp collecting and numismatic items, air filters, banknotes, etc. *(3,5,6)*.

The basic requirement for performing high quality and reliable forensic palynological analyses is the creation of comparative and reference collections of different pollen types from a certain geographical area, which includes both fieldwork and laboratory analyses. Such a collection, comprising the known forensic potential of the stored pollen, is essential for the identification of forensic or control pollen samples. To that end, simultaneously with collecting pollen, it is necessary to determine the corresponding floristic composition (so-called palynological flora) of the investigated area in order to avoid errors in making a conclusion on the "pollen traces" on the way to/from as well as at the crime scene itself *(5,6)*.

After it was discovered in the 1950s that pollen can serve as evidence that might connect a suspect to a crime scene, forensic palynology started developing as a discipline that studies pollen and spores for the purpose of criminal investigations, court proceedings, etc. *(4)*. The first recorded data of pollen analysis in a legal expertise date back to 1959; the first case occurred in Austria, another one in Sweden. Both were related to the homicide and disappearance of a victim and were solved thanks to forensic palynology *(7)*. Later on, the successful forensic application of palynological research has so far been recorded in cases the most serious criminal acts, such as homicide *(7)*, rape *(8)*, armed robbery, forgery, fraud, drug dealing *(9,10)*, war crimes and mass grave victims *(11)*, plane accidents *(12)*, document counterfeiting *(13)*, etc. Additionally, in recent times, an innovative approach to forensic palynology arose, namely to study pollen as possible evidence in legal cases in advance, e.g., without a specific criminal case (14,15,16), which has expanded the perspectives and possibilities of utilising forensic palynology. In order to avoid the contamination of pollen samples, two requirements should be met: (i) the palynologist should be among the first persons to arrive at the crime scene, and (ii) the laboratory for analysing and storing pollen samples should be sterile and isolated from all other rooms *(17,18)*. It is important to note that standard routine forensic analyses of pollen rely on light microscopy, while only critical determinations use electron microscopy *(5)*.

Routine forensic palynological analyses have already been carried out in New Zealand for more than 30 years, with increased application in Australia, the US, and some European countries (for example, the UK and Austria). In other countries, experts are aware of the importance of palynology in forensics, but its application is not yet current *(17,18)*.

Sporadic palynological studies on pollen and spores in Croatia started in the 1960s, related to the analysis of pollen in the air – aerobiology (*19*). More recently, wider applications of palynological methods have also been used for other purposes, e.g., in melisopalynology *(20)*, evolutionary taxonomy *(21)*, and vegetation history *(22)*. However, forensic palynological studies have not yet been conducted in Croatia and we have still not had the opportunity to apply forensic palynology in practice, with the possibility of applying the obtained results in legal cases. However, what we are able to do currently is either theoretical research on pollen traces *(14–16)* or practical preparation for future applications of forensic palynology (e.g., the preparation of comparative and/or reference palynological collection(s) and the determination of the forensic potential of pollen in a specific area). Therefore, the aim of this work was: (i) to create and establish an initial palynological collection, first for a limited area within Croatia, which could be used as a comparative (and in the future, reference) palynological collection; (ii) to determine and establish the forensic potential of the collected pollen.

As no floristic surveys have ever been conducted for the town of Donja Stubica (Figure 1), this area was considered suitable for our investigation, e.g., to create a model of a forensic palynological collection of pollen samples from a specific and restricted area. Our study began within the master's thesis of the first author *(6)* but was expanded in 2021.

MATERIAL AND METHODS

The small rural town of Donja Stubica is located in the southern part of the Krapina-Zagorje County and stretches over 44.6 km^2, from the Medvednica ridge and its northern slopes to the valley of the Krapina river (Figure 1). It covers part of Stubica's foothills within the Nature

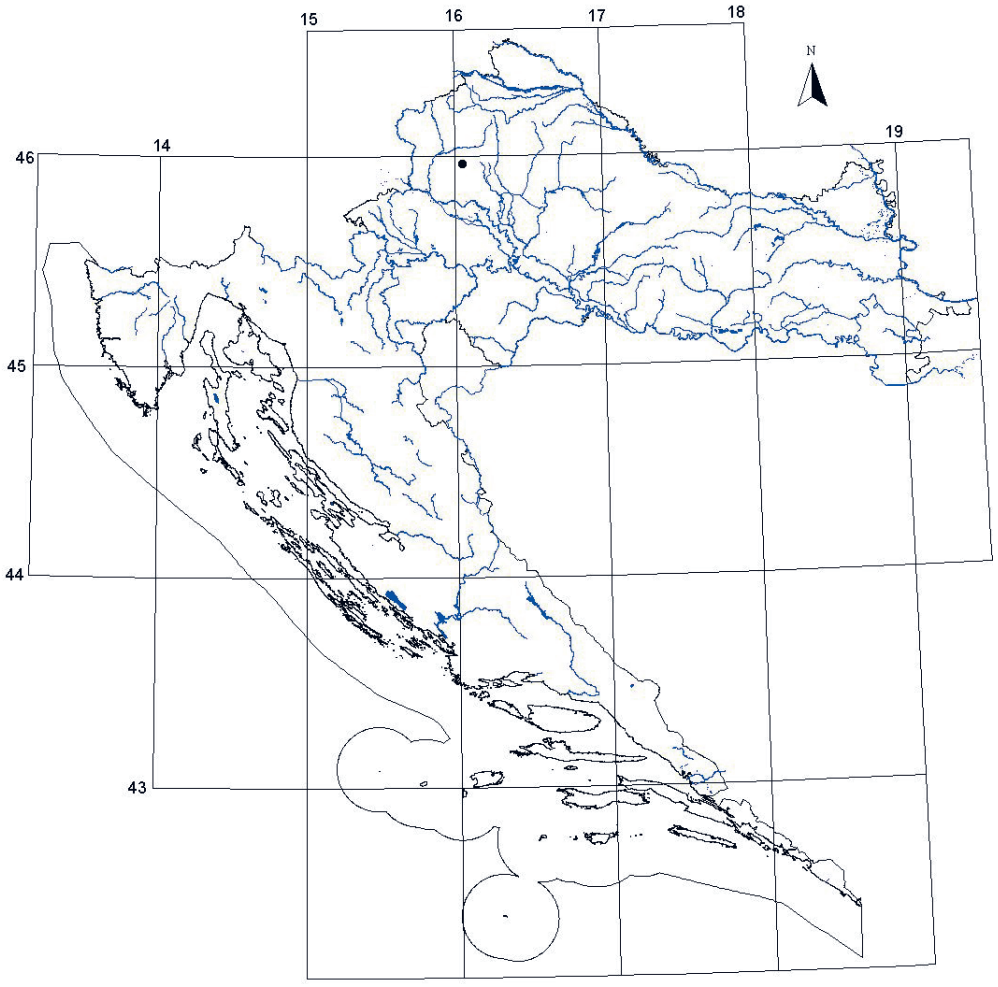

Figure 1. *The position (black dot) of the researched area of the town of Donja Stubica in Croatia.*

Park Medvednica and the hilly area between the valley of Vukšenac spring and the lowlands of Krapina river *(23)*. The biological diversity of the relevant natural habitats consists of forests, hills and valley meadows, natural courses of springs with relatively preserved typical flora and fauna of watercourses, while forests prevail within the boundaries of the Medvednica Nature Park. Meadows are significantly less represented than forests, they stretch along the hilly and valley watercourses, and are used more often as hay meadows, and less as pastures *(23)*.

The fieldwork was carried out during the 2005-2007 vegetation seasons, with additions in 2021, in the area of the town of Donja Stubica, where ten representative habitats were selected and investigated. (Table 1).

Fieldwork included collecting plant vouchers in blossom (an herbarium sample and/or photograph of each plant) and their pollen. In order to identify plant taxa, standard determination keys were used *(24,25)*, and the nomenclature of plant taxa followed the Flora Croatica Database *(26)*. The collected pollen samples (anthers / flowers / inflorescences) were deposited in paper bags and supplemented with small silica gel bags. For light microscopy, pollen grains were acetolysed according to *(14)* and slides were examined by light microscopes (an inverted Zeiss Axiovert 200 and fluorescence Olympus BH2) up to 600× magnification and photographed.

Table 1. *List of selected habitats for sampling plant taxa and pollen samples in the area of the town of Donja Stubica with the corresponding geographic coordinates.*

Sites with different habitats	Latitude	Longitude
Settlement T. Radić	5575223	5094322
Hruševec	5575445	5094640
Livadna street	5576747	5092826
Golubovec I	5576959	5093474
Golubovec II	5577119	5093234
Pustodol Il	5574930	5093166
Pustodol II	5574967	5093059
Grobljanska street I	5575147	5093458
Grobljanska street II	5575199	5093241
Obrtnička street	5575697	5092849

Samples of pollen grains were analysed by basic pollen features (shape, size, and apertures). Additionally, samples of pollen grains of the genus *Iris* were analysed by scanning electron microscopy (SEM) in order to screen for differences (especially in pollen sculpture) between them at a higher resolution. For SEM microscopy, pollen grains were prepared according to *(27)* and analysed in both hydrated and dry conditions (mounted on stubs without any preparation), while all types of samples were sputter coated with gold. The observations were made with a JEOL JSM-T 300 scanning electron microscope at the University of Vienna, Department of Structural and Functional Botany.

The pollen description terminology followed *(1,2)*. The mode of pollen dispersal and forensic potential of pollen grains was determined according to *(3)*.

All of the details of individual pollen grain features are not presented and analysed here (especially the structure of the exine, which is difficult to classify and analyse statistically due to numerous variations and transitional forms) but are available in *(6)*. Each microscopic slide of the pollen grains was associated with a photograph and plant voucher and deposited at the University of Zagreb, Faculty of Science, Department of Biology.

RESULTS AND DISCUSSION

During our research of different habitats in the area of Donja Stubica, a total of 141 representative plant taxa were collected and herborized (Supplementary 1). For each taxon, pollen samples were collected, and pollen slides were prepared, and the pollen types of the collected plants were recorded and analysed (Supplementary 1). Among the recorded plants, Dicotyledons prevailed with 111 taxa belonging to 38 families, whereas Monocotyledons were represented with 30 taxa belonging to seven families *(26)* (Supplementary 1). Among Dicotyledons, the families Asteraceae, Lamiaceae, Rosaceae, and Fabaceae were the most represented, while the Poaceae family was the most numerous among the Monocotyledons. These data were in accordance with the results of the floristic analysis of the neighbouring areas in Hrvatsko Zagorje *(28)*, as well as in Zagreb *(29)*, and indicated that we have chosen our case study area well.

Due to the vast scope of the results, only the most important pollen features of the collected plants are presented in Supplementary 1, while more detailed information is available in *(6)*.

The typical pollen features, observed during each pollen analysis, were shape and size, apertures (type and number), and sculptures of the outer pollen layer exine. Each pollen grain type was characterized by a combination of all these features, regardless of the taxonomic level for which it was specific *(1,2)*.

Regarding the shape of the pollen grains in our study (Supplementary 1, Figure 2), most of the analysed pollen grains were spheroidal (60%), followed by equal shares of oblate (18%) and prolate (18%) pollen grains. Only 3% of the collected taxa had oblate-spheroidal pollen grains, and only 1% of pollen grains were without a suitable description for their shape of pollen grains.

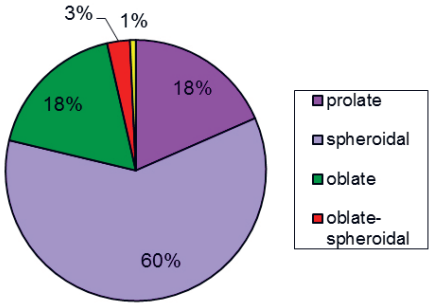

Figure 2. *Share of different shapes of pollen grains in the total sample of collected pollen in the area of the town of Donja Stubica.*

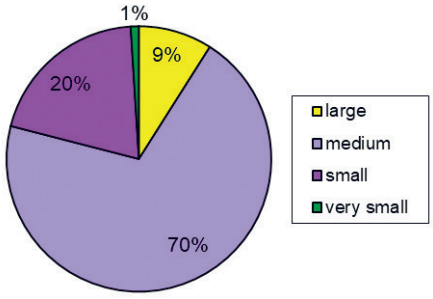

Figure 3. *Share of different sizes of pollen grains in the total sample of collected pollen in the area of the town of Donja Stubica.*

Considering the size of the pollen grains (Supplementary 1, Figure 3), most of the analysed taxa (70%) had medium-sized pollen grains. Then followed 20% of analysed taxa with small pollen grains, 9% with large pollen grains, and 1% that had very small pollen grains (Figure 6).

Regarding aperture type (Supplementary 1, Figure 4), most of the collected taxa had colporate pollen grains

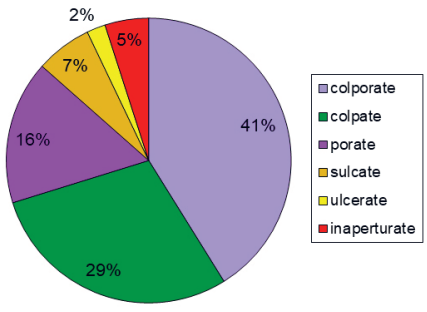

Figure 4. *Share of different aperture types of pollen grains in the total sample of collected pollen in the area of the town of Donja Stubica.*

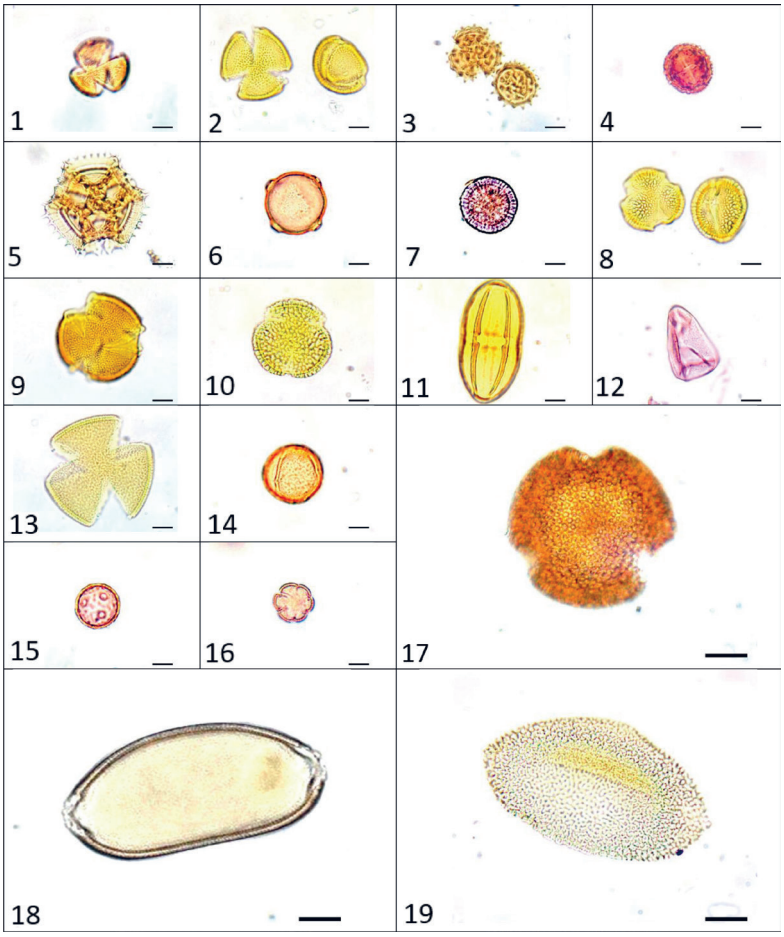

Figure 5. *Different types of pollen grains recorded in the area of the town of Donja Stubica (Light microscopy): Acer (1), Anemone (2), Senecio type (Bellis perennis) (3), Centaurea jacea type (4), Lactuca type (Cichorium) (5), Carpinus betulus (6), Daphne (7), Euonymus europaeus (8), Euphorbia (9), Ligustrum type (10), Astrantia type (Hacquetia epipactis) (11), Cyperaceae (Carex) (12), Oxalis acetosella (13), Quercus robur-pubescens type (Quercus robur) (14), Plantago lanceolata type (15), Rubiaceae (Galium) (16), Geranium (17), Colchicum autumnale (18), Iris pseudacorus type (19). Scale bar 10 µm (photos 1 to 16) or 20 µm (photos 17 to 19).*

(41%), then colpate (29%), porate (16%), sulcate (7%), inaperturate (5%), and ulcerate (2%) pollen grains.

All these analysed features of pollen grains are quite visible using a light microscope (some are shown in Figure 5) and are stored in digital form, which allows us quick access to the control samples without a re-analysis of the slides, if and when we need to consult our pollen determination in the future.

However, it should be considered that each standard pollen feature (shape and size, apertures and sculptures of exine) has its own limitations. The shape and size of pollen grains are not always completely reliable features, both due to the harmomegathy of pollen grains *(1,2)* and the possibility of their significant variability during different (pre)treatments of pollen samples *(27)*. Therefore, it is advisable to use "classes" for these features. Hence, for example, is the finding that pollen from our study was mostly spheroidal (60%) means that these pollen grains have a polar axis ± equal to the equatorial diameter, regardless of their concrete numerical dimensions *(1,2)*. Similarly, the 70% of medium-sized pollen grains in our study means that these pollen grains are between 26-50 µm in size *(1,2)*. Unlike the aforementioned, the apertures (their type and number) and ornamentation of the exine are key features for certain pollen types, although usually delimited at a higher taxonomic level than the species *(1,2)*. Apertures are the most noticeable features of pollen grains, so their shape, number, and location on the pollen grain are of great importance for the recognition of a pollen type (Figure 5).

The exine ornamentation of different pollen types can be reliably distinguished under a light microscope (Figure 5). However, the exine ornamentation of closely related taxa usually require SEM analysis for easier recognition. Thus, e.g., in our study, two taxa of the genus *Iris* (*I. pseudacorus* and *I. sibirica* subsp. *sibirica*), whose pollen grains belong to the same pollen type (cf. 30), were recorded. Therefore, only analysis by SEM (Scanning elec-

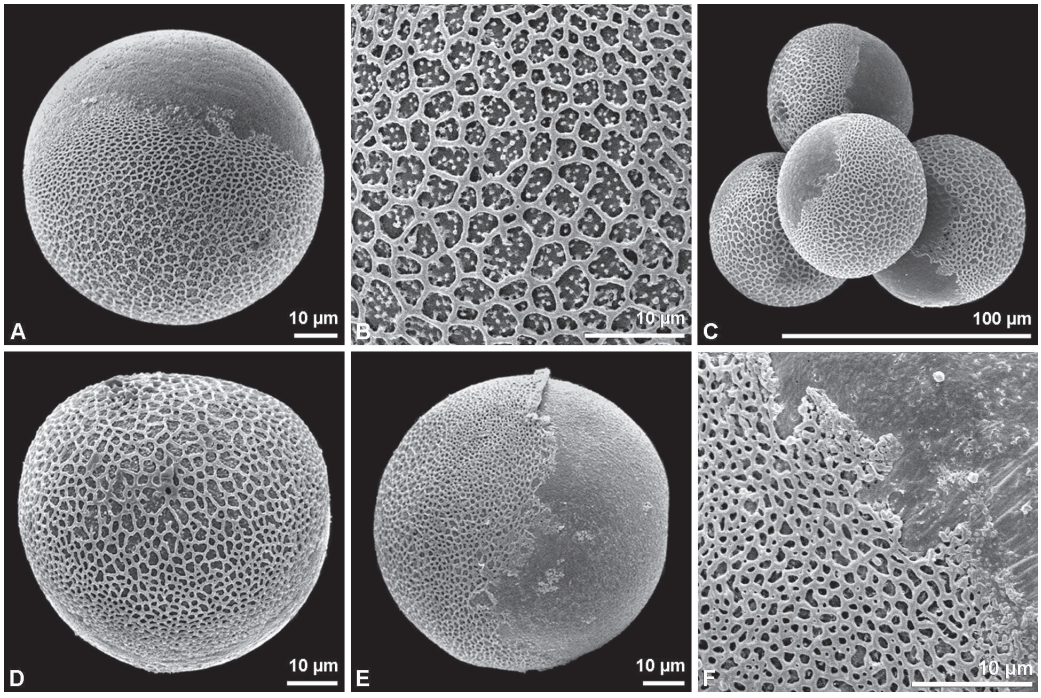

Figure 6. *An example of pollen grains of similar taxa recorded in the area of the town of Donja Stubica (SEM microscopy): A-C: I. sibirica, A – equatorial view, B – reticulate exine with free standing columellae, C – group of pollen grains; D-F: I. pseudacorus. D – equatorial view, E – equatorial view, F: exine surface with almost microreticulate sculptures.*

tron microscope) can provide us with better insight into the possible differences between the pollen grains of these two taxa: regardless of the same pollen type, we can observe differences in the structure and size of the reticulate elements of the exine sculptures (Figure 6).

Still, if we are not able to apply SEM analysis, knowledge about the distribution of individual plants can be helpful. More precisely, in addition to pollen production and transmission, when determining the forensic potential of the pollen of an individual plant, its area of distribution is also very important. For example, although both iris taxa inhabit wet and swampy areas, *I. pseudacorus* is widespread, while *I. sibirica* subsp. *sibirica* (which also has the IUCN status VU - vulnerable) is very rare, both in the researched area and in Croatia in general (FCD). Therefore, regardless of the similarity of pollen types, if we know where a pollen sample was taken, and we can compare it with a comparative or reference pollen sample, we can connect the pollen of a certain species of the genus *Iris* with a crime scene. So, the narrower the geographical distribution of a plant, the better its forensic potential. Therefore, due to its limited geographical distribution, the pollen of *I. sibirica* subsp. *sibirica* has greater forensic potential than the pollen of *I. pseudacorus*.

For the forensic analysis of a real crime or an experimental case, the aforementioned pollen features are important in terms of recognizing and counting individual pollen types, because they enable the palynologist to interpret the results of the expert examination.

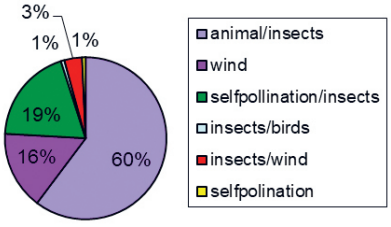

Figure 7. *Share of different modes of pollen dispersal in the total sample of collected pollen in the area of the town of Donja Stubica.*

In contrast, the feature that is usually not included in standard pollen analyses, namely, the mode of pollen dispersal, is much more important for determining the forensic potential of pollen. Results of our research showed that, according to methods of dispersal of pollen grains (Figure 7), most of collected taxa are zoogamous (60%), followed by autogamous-zoogamous (19%), anemophilous (16%), anemophilous-zoogamous (3%), insects-birds (1%) and autogamous (1%).

Consequently, our results indicated the dominance (60%) of pollen of excellent forensic potential (group IV, Supplementary 1) in the investigated area of continental Croatia (Figure 8). Furthermore, a significant number of the researched plants (23%) had a combination of two methods of pollen dispersal: with insects and possible self-pollination (19%), dispersal with insects and wind (3%), and with insects and birds (1%). Therefore, according to

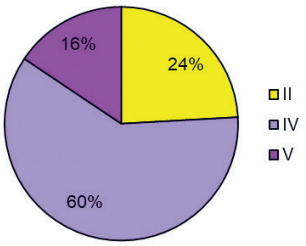

Figure 8. *Share of different forensic potentials of pollen grains in the total sample of collected pollen in the area of the town of Donja Stubica (forensic potential: II – very important; IV – excellent; V – excellent to poor, depending on the plant associations).*

their forensic potential they all belong to group II (very important forensic potential if they are present in a sample; Supplementary 1), as well as autogamous plants (1%). Additionally, 16% of the researched plants had an excellent to poor forensic potential of pollen grains, depending on the plant associations in which they live (group V, Supplementary 1).

Besides the mode of pollen dispersal, important features for the forensic potential of a pollen also include the relative level of pollen production and the preservation potential of pollen grains *(31,32)*. Plants that produce higher quantities of pollen grains have less efficient dispersal method, e.g., anemophilous plants *(3)*. The relative levels of pollen production range from <100 grain per anther for autogamous and cleistogamous plants, 100-1,000 grains per anther for zoogamous plants, and 1000-100,000 grains per anther in anemophilous plants *(3)*. Furthermore, the pollen of autogamous and cleistogamous plants can very rarely be found in the environment, far from the mother plant, as is the case with the pollen of zoogamous plants. Therefore, the presence of such pollen in analysed forensic samples provides a stronger indication related to the specific attributes of a crime site than is the case with the pollen of anemophilous plants *(33)*. The poor forensic potential of the pollen of anemophilic plants is therefore the result of very high productivity and the low weight of pollen grains, which is why they can be found in a very large area and at a great distance from the mother plant, about 25-1,000 m from a mother plant (95% grains) *(34)*. It should also be emphasized that there have been episodes of long-distance transport of anemophilic pollen by air masses recorded, at a distance of over several hundred km from the source *(35)*. The best example of the importance of having knowledge on the relative level of pollen production in forensic analysis are some species from the genus *Cannabis*, which produce up to 70,000 grains per anther *(36)*. Although certain species of this genus are often the subject of forensic examinations aimed at the detection of drug abuse, the forensic potential of its pollen is weak due to its large production. More precisely, such a large presence of *Cannabis* pollen in the air means a lower reliability rate in establishing a direct connection between a suspect and a precisely defined source of pollen dispersal (e.g., nursery).

Opposite to this, zoophilous plants, i.e., plants whose pollen grains are spread by animals (mainly insects), due to the efficiency of their method produce relatively small amounts of pollen, and these pollen grains are heavy with a complex ornamentation of the exine. Such pollen grains are well preserved in the layers of the Earth, and we can find them near the mother plant. Because of this, the finding of such pollen grains in the contested sample indicates direct contact with the plant itself or the soil in its immediate vicinity *(5,6)*.

The preservation potential of all pollen grains is generally excellent, thanks to the polymer sporopollenin which forms the exine, the outer layer of the pollen wall, whose stability and resistance are well known *(5-7)*. Even in unfavourable conditions of preservation, most pollen grains can remain preserved for several years in a layer of leaves and/or surface soil layer. For example, in the first recorded forensic application of pollen analysis mentioned earlier, the case of a man murdered in Austria, 20-million-year-old pollen grain from the Miocene was established as crucial evidence at the crime scene and demonstrated the great potential of pollen preservation. In this instance, in a limited area along the Danube banks, due to erosion pollen grains from older layers were integrated in newer layers so the referenced pollen grain ended up in the soil sample exempted for forensic analysis *(7)*.

To conclude, our collection of pollen samples with vouchers would primarily be useful as a comparative collection during identification, but the entire collection or part of it could also later be transformed into a reference collection. Reference collections are usually more organised, with data and photographs available in databases or in written or electronic form (often with identification keys) on specialized web sites. As pollen grains are nearly invisible traces that can (dis)prove a criminal suspect's connection to the crime scene, as well as confirm or deny one's alibi, the data on the forensic potential of our pollen collection can be used for analyses of pollen samples in specific cases of legal expertise in continental Croatia. However, we should also be aware of the limitations and therefore the possibility of inference errors when using palynological methods in forensics (e.g., difficulties with pollen determination, potential contamination of pollen sample, predominant specificity of pollen grains at the genus-types level, etc.). Therefore, a more authentic determination of the forensic potential of the pollen of an individual plant also requires knowledge of the distribution and biology of that plant and its pollen. In that sense, it is important to emphasize here the need for the participation of educated botanists / palynologists in forensic research in order to achieve the most accurate determination of plants and palynological samples possible, which will lead to the most correct interpretations for the purpose of legal proceedings.

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SUPPLEMENTARY 1.

The list of analysed palynological flora of the city of Donja Stubica (continental Croatia) with the data as follows: **plant names** presented alphabetically, with **family** affiliation (FCD); pollen features [33] – **shape** (P – prolate, S – spheroidal, O – oblate, NSD – no suitable description), **aperture type** (CPA – colpate, CPO – colporate, POR – porate, SUL – sulcate, ULC – ulcerate, PID – poroid; inAP – inaperturate), **approximate size** VS – very small, SM – small, M – medium, L – large); **pollen dispersal methods**: (AU – autogamous or self-pollination, ZO – zoogamous or animal/insect pollination, AN – anemophilous or wind-pollination); **forensic potential**: II – rare, but very specific if present (AU), IV – excellent (ZO), V – excellent to poor, depending on the plant associations (AN); and **level of threat**, if any (IUCN categories, 31) (VU – vulnerable, NT – near threatened).

1. *Acer campestre* L. – Aceraceae, S; 3CPA; M; AN+ZO; IV,V
2. *Achillea millefolium* L. – Asteraceae, S; 3CPO; M; ZO; IV
3. *Adoxa moschatellina* L. – Adoxaceae, S; 3CPO[3CPA]; M; AN+ZO; IV,V
4. *Ajuga reptans* L. – Lamiaceae, S; 3CPA; M; ZO; IV
5. *Alliaria petiolata* (M. Bieb.) Cavara et Grande – Brassicaceae P; 3CPO; M; ZO; IV
6. *Allium ursinum* L. – Liliaceae, O; SUL; M; ZO; IV
7. *Alopecurus pratensis* L. – Poaceae, S; ULC; M; AN;V
8. *Anemone nemorosa* L. – Ranunculaceae, S; 3CPA; SM; ZO; IV
9. *Anthoxanthum odoratum* L. – Poaceae, P; ULC; M, AN; V
10. *Aposeris foetida* (L.) Less. – Cichoriaceae, S; 3CPO; M; ZO; IV
11. *Arum maculatum* L. – Araceae, S; inAP; SM-M; ZO; IV
12. *Bellis perennis* L. – Asteraceae, S; 3CPO; SM; ZO; IV
13. *Betonica officinalis* L. – Lamiaceae, S; 3CPA; M; ZO; IV
14. *Calamintha vulgaris* (L.) Druce – Lamiaceae, O; 6CPA; M-L; ZO; IV
15. *Caltha palustris* L. – Ranunculaceae, S; 3CPA; M; ZO; IV
16. *Campanula trachelium* L. – Campanulaceae, S; 3POR; M; AU+ZO; II, IV
17. *Cardamine bulbifera* (L.) Cr. – Brassicaceae, P; 3CPA; M; AU+ZO; II, IV
18. *Cardamine hirsuta* L. – Brassicaceae, S; 3CPA; SM; ZO; IV
19. *Cardamine pratensis* L. – Brassicaceae S; 3CPA; M; ZO; IV
20. *Carex acuta* L. – Cyperaceae, P; PID; M; AN; V
21. *Carex distans* L. – Cyperaceae, P; PID; M; AN; V
22. *Carex hirta* L. – Cyperaceae, P; PID; M; AN; V
23. *Carex pilulifera* L. – Cyperaceae, P; PID; M; AN; V; NT
24. *Carex sylvatica* Huds. – Cyperaceae, P; PID; M; AN; V
25. *Carex vulpina* L. – Cyperaceae, P; PID; M; AN; V
26. *Carpinus betulus* L. – Betuaceae, S; 4POR; M; AN; V
27. *Centaurea jacea* L. – Asteraceae, S; 3CPO; M; AU+ZO; II, IV
28. *Chamomilla recutita* (I.) Rauschert – Asteraceae, S; 3CPO; M; ZO; IV
29. *Chelidonium majus* L. – Papaveraceae, S; 3CPA; M; ZO; IV
30. *Cichorium intybus* L. – Cichoriaceae, S; 3CPO; M; ZO; IV
31. *Colchicum autumnale* L. – Liliaceae, O; diP; L; ZO; IV
32. *Cornus sanguinea* L. – Cornaceae, S; 3CPO; M; AU+-ZO; II, IV
33. *Corydalis bulbosa* (L.) DC. – Fumariaceae, S; 6CPA; M-L, ZO; IV
34. *Crataegus monogyna* Jacq. – Rosaceae, O; 3CPO; L; ZO; IV
35. *Cruciata glabra* (L.) Ehrend. – Rubiaceae, S; 6CPA; SM; ZO; IV
36. *Cruciata laevipes* Opiz – Rubiaceae, S; 6CPA; SM; ZO; IV
37. *Cyclamen purpurascens* Mill. – Primulaceae, S; 3CPO; SM; AU+ZO; II, IV; NT
38. *Cynosurus cristatus* L. – Poaceae, S; ULC; M; AN;V
39. *Dactylis glomerata* L. – Poaceae, S; ULC; M; AN;V
40. *Daphne mezereum* L. – Thymelaeaceae, S; POR; M; AU+ZO; II, IV; NT
41. *Daucus carota* L. – Apiaceae, P; 3CPO; M; ZO; IV
42. *Deschampsia cespitosa* (L.) P. Beauv. – Poaceae, S; ULC; M; AN;V
43. *Diplotaxis muralis* (L.) DC – Brassicaceae, S; 3CPA; M-L; ZO; IV
44. *Epilobium hirsutum* L. – Onagraceae, NSD; 3POR; L; ZO; IV
45. *Erigeron annus* (L.) Pers. – Asteraceae, S; 3CPO; SM; ZO; IV
46. *Erythronium dens-canis* L. – Liliaceae, O; SUL; M-L; ZO; IV
47. *Euonymus europaeus* L. – Celastraceae, O-S; 3CPO; M; ZO+ B; IV
48. *Euphorbia angulata* Jacq. – Euphorbiaceae, S; 3CPO; M; ZO; IV
49. *Euphorbia epithymoides* Kern. – Euphorbiaceae, S; 3CPO; M; ZO; IV
50. *Festuca pratensis* Huds. – Poaceae, P; ULC; M, AN; V
51. *Filipendula ulmaria* (L.) Maxim. – Rosaceae, S; 3CPO; SM; ZO; IV
52. *Filipendula vulgaris* Moench. – Rosaceae, S; 3CPO; SM; ZO; IV
53. *Fragaria moschata* Duchesne – Rosaceae, O; 3CPO; M; ZO; IV

54. *Fragaria vesca* L. – Rosaceae, O; 3CPO; SM; ZO+AU; II, IV
55. *Gagea lutea* (L.) Ker. G. – Liliaceae, O; SUL; L; ZO; IV
56. *Galanthus nivalis* L. – Amaryllidaceae, O; SUL; M; ZO; IV; LC
57. *Galeopsis speciosa* Mill. – Lamiaceae, S; 3CPA; M; ZO; IV
58. *Galium mollugo* L. – Rubiaceae, S; 6CPA; SM; ZO; IV
59. *Galium odoratum* (L.) Scop. – Rubiaceae, O; 6CPA; SM; ZO; IV
60. *Gaudinia fragilis* (L.) P. Beauv. – Poaceae, S; ULC; M; AN;V
61. *Geranium phaeum* L. – Geraniaceae, S; 3CPO; L; ZO; IV
62. *Geranuim robertianum* L. – Geraniaceae, O; 3CPO; M; ZO; IV
63. *Glechoma hederacea* L. – Lamiaceae, O; 6CPA; M; ZO; IV
64. *Glechoma hirsuta* Waldst. et Kit. – Lamiaceae, O; 6CPA; L; ZO; IV
65. *Hacquetia epipactis* (Scop.) DC. – Apiaceae, P; 3CPO; M; ZO+AU; IV, II
66. *Hepatica nobilis* Schreb. – Ranunculaceae, S; 3CPA; M; ZO; IV
67. *Holcus lanatus* L. – Poaceae, S; ULC; M, AN; V
68. *Iris pseudacorus* L. – Iridaceae, O; SUL; L; ZO; IV
69. *Iris sibirica* L. subsp. *sibirica* – Iridaceae, O; SUL; L; ZO; IV, VU
70. *Juncus effusus* L. – Juncaceae, NSD; ULC; SM; AN;V
71. *Knautia arvensis* (L.) Coult. – Dipsacaceae, O; 3CPO; L; AU+ZO; II, IV
72. *Knautia drymeia* Heuff. – Dipsacaceae, O; 3POR; L; ZO; IV
73. *Lamium galeobdolon* (L.) L. – Lamiaceae, O-S, 3CPA; SM-M; ZO; IV
74. *Lamium orvala* L. – Lamiaceae, S; 3CPA; SM-M; ZO; IV
75. *Lamium purpureum* L. – Lamiaceae, S; 3CPA; M; ZO; IV
76. *Lathyrus vernus* (L.) Bernh. – Fabaceae, P; 3CPO; M; ZO; IV
77. *Leucanthemum vulgare* Lam. – Asteraceae, S; 3CPO; SM; AU+ZO; II, IV
78. *Leucojum vernum* L. – Amaryllidaceae, O; SUL; M; AU+ZO; II, IV
79. *Ligustrum vulgare* L. – Oleaceae, O-S; 3CPO; M; AU+-ZO; II, IV
80. *Linaria vulgaris* Mill. – Scrophulariaceae, S; 3CPO; SM; ZO; IV
81. *Lithospermum purpurocaeruleum* L. – Boraginaceae P; 4CPA; SM; ZO; IV
82. *Lonicera caprifolium* L. – Caprifoliaceae, S; 3CPO; L; ZO; IV
83. *Lotus corniculatus* L. – Fabaceae, S; 3CPO; SM; ZO; IV
84. *Luzula campetris* (L.) DC. – Juncaceae, O; ULC; M; AN; V
85. *Luzula pilosa* (L.) Wild. – Juncaceae, O; ULC; SM; AN; V
86. *Lychnis flos cuculi* L. – Caryophyllaceae, S; pPO; M; AU+ZO; II, IV
87. *Lysimachia vulgaris* L. – Primulaceae, S; 3CPO; SM-M; ZO; IV
88. *Myosotis scorpioides* L. – Boraginaceae, P; 3CPO; VSG; ZO; IV
89. *Ononis spinosa* ssp. *arvensis* (L.) Greuter et Burdet – Fabaceae, P; 3CPO; SM; ZO; IV
90. *Oxalis acettosella* L. – Oxalidaceae, S; 3CPA; M; AU+-ZO; II, IV
91. *Papaver rhoeas* L. – Papaveraceae, S; 3CPA; M; AN+-ZO; IV, V
92. *Phleum pratense* L. – Poaceae, P; ULC; M, AN; V
93. *Plantago lanceolata* L. – Plantaginaceae, S; pPOR; M; AN+ZO; IV, V
94. *Plantago maior* L. – Plantaginaceae, S; pPOR; SM; AN; V
95. *Poa annua* L. – Poaceae, S; ULC; SM, AN; V; LC
96. *Polygala comosa* Schkuhr – Polygalaceae, S; CPO; M-L, ZO, IV
97. *Polygonatum odoratum* (Mill.) Druce. – Liliaceae, S; SUL; L; AU+ZO; II, IV
98. *Polygonum aviculare* L. – Polygonaceae, P; 3CPO; M; AU; II
99. *Potentilla erecta* (L.) Rauschel – Rosaceae, O; 3CPO; M; ZO; IV
100. *Potentilla reptans* L. – Rosaceae, O; 3CPO; M; ZO; IV
101. *Primula vulgaris* Huds. – Primulaceae, S; CPA; SM; ZO; IV
102. *Prunus avium* L. – Rosaceae, O; 3CPO; M; AU+ZO; II, IV
103. *Prunus padus* L. – Rosaceae, O; 3CPO; M; ZO; IV
104. *Prunus spinosa* L. – Rosaceae, S; 3CPO; M; ZO; IV
105. *Pulmonaria officinalis* L. – Boraginaceae P; 4CPO; M; ZO; IV
106. *Quercus robur* L. – Fagaceae, S; 3CPA; M; AN; V
107. *Ranunculus acris* L. – Ranunculaceae, S; 6CPA; SM; ZO; IV
108. *Ranunculus ficaria* L. – Ranunculaceae, S; 3CPA; M; ZO; IV
109. *Ranunculus lanuginosus* L. – Ranunculaceae, S; CPA; M; ZO; IV
110. *Robinia pseudacacia* L. – Fabaceae, S; 3CPO; M; ZO; IV
111. *Rubus idaeus* L. – Rosaceae, O-S; 3CPO; SM; ZO; IV
112. *Salix caprea* L. – Salicaceae, S; 3CPA; SM; ZO+AN; IV, V
113. *Salix fragilis* L. – Salicaceae, S; 3CPA; M; ZO; IV
114. *Sambucus nigra* L. – Caprifoliaceae, S; 3CPO; SM; AU+ZO; II, IV

115. *Scilla bifolia* L. – Liliaceae, O; SUL; M; ZO; IV
116. *Sedum acre* L. – Crassulaceae, S; 3CPO; SM; AU+ZO; II, IV
117. *Senecio erraticus* Bert. – Asteraceae, S; 3CPO; M; ZO; IV
118. *Silene latifolia* Poir.ssp.*alba* (Mill.) Grauter et Bourdet – Caryophyllaceae, S; pPO; M; ZO; IV
119. *Stachys sylvatica* L. – Lamiaceae, S; 3CPA; M; AU+ZO; II, IV
120. *Stellaria graminea* L. – Caryophyllaceae, S; pPO; M; AU+ZO; II, IV
121. *Stellaria holostea* L. – Caryophyllaceae, S; pPO; M; AU+ZO; II, IV
122. *Stellaria media* (L.) Vill. – Caryophyllaceae, S; pPO; M; AU+ZO; II, IV
123. *Symphytum officinale* L. – Boraginaceae P; CPO; M; AU+ZO; II, IV
124. *Symphytum tuberosum* L. – Boraginaceae P; CPO; M; ZO; IV
125. *Tanacetum vulgare* L. – Asteraceae, S; 3CPO; M; ZO; IV
126. *Taraxacum officinale* Web. – Cichoriaceae, S; 3CPO; M; ZO; IV
127. *Thymus pulegioides* L. – Lamiaceae, S; 3CPA; SM-M; ZO; IV
128. *Trifolium campestre* Schreber – Fabaceae, P; 3CPO; M; ZO; IV
129. *Trifolium pratense* L. – Fabaceae, S; 3CPO; M; ZO; IV
130. *Tussilago farfara* L. – Asteraceae, S; 3CPO; M; ZO; IV
131. *Valeriana officinalis* L. – Valerianaceae, S; 3CPA; M; AU+ZO; II, IV
132. *Veronica chamaedrys* L. – Scrophulariaceae, S; 3CPA; M; AU+ZO; II, IV
133. *Veronica officinalis* L. – Scrophulariaceae, S; 3CPA; M; ZO; IV
134. *Veronica persica* Poir. – Scrophulariaceae, S; 3CPA; M; ZO; IV
135. *Viburnum lantana* L. – Caprifoliaceae, S; 3CPO; M; ZO; IV
136. *Viburnum opulus* L. – Caprifoliaceae, S; 3CPO; M; AU+ZO; II, IV
137. *Vicia cracca* L. – Fabaceae, P; 3CPO; M; ZO; IV
138. *Vicia sativa* L. ssp. *cordata* (Hope) Batt. – Fabaceae, P; 3CPO; M; ZO; IV
139. *Vicia sepium* L. – Fabaceae, P; 3CPO; M; ZO; IV
140. *Vinca minor* L. – Apocynaceae, O; 3CPO; L; AU+ZO; II, V
141. *Viola alba* Besser – Violaceae, S; 3CPO; M; ZO; IV