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Molecular detection of the botanical origin of pollen in honey bee-collected pellets: a comparison of methods

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Molecular detection of the botanical origin of pollen in honey bee-collected pellets: a comparison of methods

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ABSTRACT

Identification of botanical origin of mixed pollen samples has several applications, including assessment of plant-pollinator interactions, botanical origin of honey, monitoring of pesticide use, monitoring of allergy-related airborne pollen sources, among others. Such applications, however, have previously been limited to conventional pollen identification via light microscopy, which usually has low taxonomic resolution and requires expert knowledge. One alternative for botanical identification of mixed pollen samples is to use of DNA metabarcoding high throughput sequencing (HTS), which could overcome these drawbacks. Recent studies demonstrate that the nuclear barcoding marker *ITS2* (internal transcribed spacer 2 region of nuclear ribosomal DNA) can be amplified from DNA extracted from mixed pollen samples. The aim of this study was to compare a variety of methods of storage/transportation and DNA extraction that ensure good DNA yield and quality appropriate for botanical identification of mixed pollen samples by means of a DNA metabarcoding approach, combining the amplification of *ITS2* with HTS. In the context of the international project "INSIGNIA: environmental monitoring of pesticide use through honeybees", mixed pollen samples were collected from traps set up in apiaries from several European countries, stored by beekeepers and later transported to the laboratory of CIMO for identification of plant taxa and inference of relative abundances. Four methods of genomic DNA isolation (NucleoSpin Food kit, GF-1 Plant kit, HigherPuritykit, and CTAB-PVP) were compared regarding DNA yield and purity by means of spectrophotometry and standard gel electrophoresis. Additionally, four storage/transportation methods of trap-collected pollen samples (freezing at -20 ℃, drying at 25℃ for 2 days, drying with silica, and placing in ethanol) were compared to assess their impact on the quality and quantity of extracted DNA. The results demonstrated the superior efficacy of the NucleoSpin DNA extraction method. The different storage/transportation conditions of pollen samples were compared for their impact on DNA quality and quantity using the NucleoSpin as the DNA extraction method. The results showed that the DNA extracted from the pollen samples placed in ethanol had the best quality/yield compared to the DNA extracted from the other samples with different storage conditions. Two primer pairs targeting *ITS2* region ITS-S2F/ITS4R and ITS-u3/ITS-u4, were employed to identify plant taxa via metabarcoding HTS. The number of taxa identified in common using these two primers were 48 families, 118 genera, and 204 species, corresponding to 87.2 % , 79.5%, and 68.7%, respectively.The results of identification of taxa we present very similar results, making comparisons

difficult, with a slight difference in the number of taxa (ITS-u3/ITS-u4 with higher number of identified taxa) and the abundance (ITS-S2F/ITS4R with higher abundance of taxa identified). This study thus offers improvements in the laboratory workflow ensuring a good DNA quantity and quality for downstream HTS applications.

Keywords: pollen identification, DNA extraction, storage methods, *ITS2*, DNA metabarcoding, HTS

RESUMO

A identificação da origem botânica de amostras de pólen tem várias aplicações, incluindo avaliação das interações planta-polinizador, origem botânica do mel, monitorização do uso de pesticidas, monitorização de fontes de pólen do ar causadoras de alergias, entre outras. Tais aplicações, no entanto, têm sido limitadas pela identificação convencional de pólen por microscopia óptica, que geralmente possui baixa resolução taxonómica e requer conhecimento especializado. Uma alternativa para a identificação botânica de amostras mistas de pólen é o uso de "DNA metabarcoding high throughput sequencing (HTS)", que pode superar essas desvantagens. Estudos recentes mostram que o código de barras nuclear ITS2 (região espaçadora interna transcrita 2 do DNA ribossómico nuclear) pode ser amplificado a partir de DNA extraído de amostras mistas de pólen. O objetivo deste estudo foi comparar uma variedade de métodos de armazenamento/transporte e extração de DNA que garantam um bom rendimento e qualidade do DNA adequados para a identificação botânica de amostras de pólen misto por meio de uma abordagem de DNA metabarcoding, combinando a amplificação de ITS2 com HTS. No contexto do projeto internacional "INSIGNIA: monitorização ambiental do uso de pesticidas através das abelhas", foram colhidas amostras de pólen a partir de capta polens montados em apiários em vários países da Europa, armazenadas pelos apicultores e posteriormente foram transportadas para o laboratório do CIMO para identificação botânica e inferência das abundâncias relativas. Quatro métodos de isolamento de DNA genómico (NucleoSpin Food kit, GF-1 Plant kit, HigherPuritykit e CTAB-PVP) foram comparados quanto ao rendimento e pureza do DNA por meio de espectrofotometria e eletroforese em gel de agarose. Adicionalmente, quatro métodos de armazenamento/transporte de amostras de pólen (congelamento a -20 ° C, secagem a 25 ° C por 2 dias, secagem com sílica e colocação em etanol) foram comparados para avaliar o seu impacto na qualidade e quantidade do DNA extraído. Os resultados demonstraram a superioridade do método de extração de DNA NucleoSpin. As diferentes condições de armazenamento/transporte das amostras de pólen foram comparadas quanto ao seu impacto na qualidade e quantidade do DNA, usando o NucleoSpin como método de extração de DNA. Os resultados mostraram que o DNA extraído das amostras de pólen colocadas em etanol apresentou a melhor relação qualidade/rendimento comparado com o DNA extraído das outras amostras submetidas a diferentes condições de armazenamento. Dois pares de primers da região ITS2, ITS-S2F/ITS4R e ITS-u3/ITS-u4, foram utilizados para identificar os taxa representados nas amostras de pólen através do método DNA metabarcoding HTS. O número de taxa

identificados em comum usando estes dois pares de primers foi de 48 famílias, 118 géneros e 204 espécies, correspondendo a 87,2%, 79,5% e 68,7%, respectivamente. A análise dos resultados sugere que os dois pares de primers são muito semelhantes, com uma pequena diferença no número de taxa (ITS-u3/ITS-u4 com maior número de taxa identificados) e na abundância (ITS-S2F/ITS4R com maior abundância de taxa identificados). Este estudo contribuiu para a melhoria do fluxo de trabalho laboratorial garantindo uma boa quantidade e qualidade do DNA com vista a aplicações HST a jusante.

Palavras-chave: identificação de pólen, extração de DNA, métodos de armazenamento, ITS2, DNA metabarcoding, HTS

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I. Introduction

1. Framework

Insect pollination, also known as Entomophily, is an indispensable process in the functioning of natural ecosystems (Saunders, 2018). It is also vital for agricultural systems, with 75% of plant species benefiting from insect pollinators to assure their reproduction (Goulson et al., 2015). The benefit is reciprocal as flowers provide insect pollinators with rich nutrients (nectar and/or pollen), which are crucial for their survival. Sugary nectar supplies pollinators with carbohydrates while pollen offers proteins, fats, vitamins and minerals (Campos et al., 2008).

Among the numerous insect species that act as pollinators, bees are the most important and the predominant ones (Potts et al., 2010). While bees are crucial for the environmental balance, they are also important for the economy. It is estimated that honey bees, together with bumblebees and other wild bee species**,** bring out at least 22 billion € each year to the European agriculture industry (European Commission, 2017). The economic importance of pollination, and its esthetical and ethical values, makes it clear that conservation of pollination systems is an important priority. Several factors intervene in maintaining the balance of pollination systems, including health of pollinators, climate change, annual changes in local flora and flowering phenology (Linskens & Jorde, 1997). Identification of the botanical origin of pollen collected by bees has both fundamental and practical applications. At a more fundamental level, it can be used to help disentangling ecological processes such as pollinator-plant interactions and to understand foraging biology of the bees. Currently, this is considered of high relevance since pollinators are increasingly being affected by a variety of human activities and, consequently, there is a strong demand for management programs that would enhance pollinators populations. However, for the successful implementation of such programs, the knowledge about the interaction of pollinators and pants is crucial (Saunders, 2018). At a more practical level, assessing the botanical origin of polled can be used in the authentication of apicultural products (Prosser & Hebert, 2017) and for monitoring allergy-related airborne pollen sources (Kraaijeveld et al., 2015).Until recently, identification of mixed pollen samples, either extracted from honey or collected in pollen traps, relied on morphological traits of pollen exine. The problem is that this method is very time-consuming, it requires an in-depth knowledge of the bioregions foraged by

honey bee colonies and plant taxa of interest, but above all it lacks resolution for species-level identification in many plant taxa (Bell et al. 2015; Sickel et al., 2015).

Recent advances in molecular technologies, particularly next generation sequencing (NGS), provide a powerful alternative for identifying the botanical origin of pollen grains in mixed pollen samples. Specifically, DNA metabarcoding using a high throughput sequencing (HTS) approach has been proposed to tackle the challenge of identifying plant taxa contained in mixed pollen samples. Several chloroplastic and nuclear genes have been proposed as barcodes, namely *rbcL, matK, trnH-psbA* and *ITS* (Bell et al., 2017). In this study the nuclear barcoding marker *ITS2* (internal transcribed spacer 2 region of nuclear ribosomal DNA) will be used as an efficient alternative method to identify the botanical origin of pollen in honey bee-collected pellets.

2. Objectives

This study will be developed in the framework of the international project "INSIGNIA –Environmental monitoring of pesticide use through honeybees" .In this project, mixed pollen samples will be collected from pollen traps across Europe using an apiculturist citizen science approach. Pollen samples will be stored by beekeepers during the collecting season and later transported to the laboratory of CIMO for identification of plant taxa and relative abundances via DNA metabarcoding high throughput sequencing (HTS) with *ITS2*. A variety of storage and transportation methods of pollen samples will be compared for their impact on DNA quality and quantity. These are critical for downstream DNA metabarcoding HTS with ITS2 and therefore for successful botanical identification of mixed pollen samples. In this context, the objectives of this study are two-fold:

1) To compare a variety of extraction methods for DNA quality and yield;
2) To compare different storage and transportation methods of mixed pollen samples for their impact on the DNA quality and yield obtained using the isolation method selected in objective 1.
3) To compare two primer pairs targeting the *ITS2* nuclear region, ITS-S2F/ ITS4R and ITS-u3/ITS-u4, via HTS metabarcoding, regarding botanical identification of mixed pollen samples collected across a wide geographical area in Europe.

In accomplishing these objectives, the following questions will be addressed:

1) What is the pollen extraction method that ensures a good quantity and quality

of DNA for downstream NGS applications?

2) Which extraction method produces the most accurate estimates of relative taxa abundances in the mixed pollen samples?
3) Which method should be recommended to beekeepers for storing and transporting pollen samples collected from traps?
4) Which *ITS2* primer pair succeeded in identifying taxa via HTS metabarcoding?

II. Literature review

1. Insect pollination

The goal of all living beings is to ensure its continuity. For plants this can be achieved by seeds formation that contains genetic information to create a new generation of plants. The phenomenon that leads to the formation of seeds is called fecundation, which consists in transferring the pollen grains from the male anther to the female stigma of the flower. Many natural vectors, such as water, wind and animals, transport the pollen from one flower to another. Pollinators are the set of animals that perform this transfer owing to their hairy bodies so that the pollen can adhere to it (Kleijn et al., 2015). The insects are the most important pollinators of the animal kingdom, with the remaining pollinators, other than insects, being birds and bats, which represent only 10% of pollinating animals (Figure 1; Hoshiba & Sasaki, 2008). Within the class Insecta, several studies have shown that the majority of pollinators are bees, among which honey bees, bumblebees, orchard bees, squash bees, and solitary bees are the most important species involved in pollination. Among these, honey bees have an important role in agriculture as they are able to increase yield in 96% of many animal-pollinated crops (Potts et al., 2010).

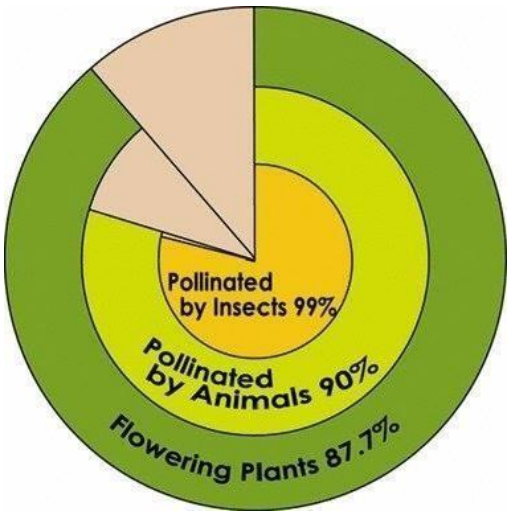


Figure 1: Percentage of plants pollinated by insects (Source: Hoshiba & Sasaki, 2008).

2. Honey Bees

Honey bees, *Apis mellifera* L., are hymenopterans of the Apidae family. They have a high level of organization of animal sociality, being characterized by cooperative brood care living within colonies composed of a reproductive female (the queen), a non-reproductive group of diploid females (the workers), and the haploid males (drones).On the first three weeks of their lives, the young worker bees carry out several tasks within the hive. They are responsible for feeding and cleaning larvae, cleaning the comb cells, building comb, guarding the colony, tending the queen, accepting pollen from foragers, storing, and packing pollen, among other tasks. As the workers become older, the glands that produce larval food and wax degenerate and they leave the brood nest and start its integration into the life of a forager. Forager bees collect water, propolis, nectar and pollen from different flowers during the flowering seasons (Wright et al., 2018). The nectar carried by foragers is received by younger workers and is stored as honeywhereas pollen will be transformed into bee bread. Both will be used to feed the larvae(Figure 2).

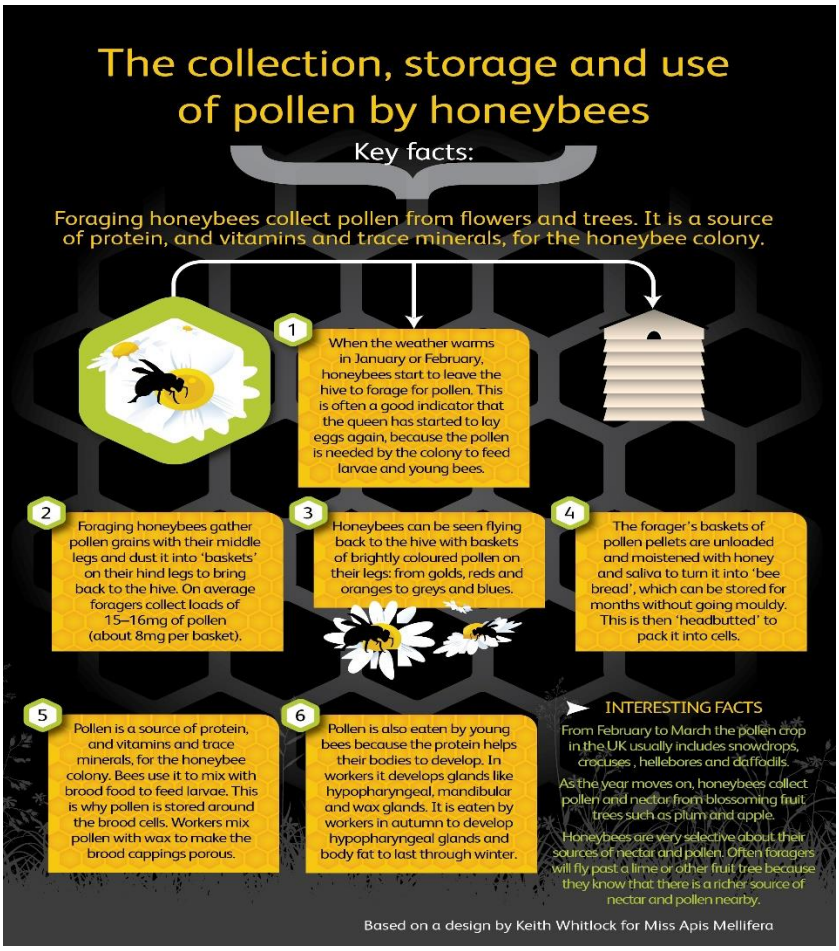


Figure 2: The flow of food in a honey bee colony (Source: https://missapismellifera.com).

3. Pollen diversity

Pollen is the male gametophyte (reproductive units) produced by the anthers of flowers. Pollen grains may present different sizes, shapes, and colors, depending on the plant source. To identify pollen grains there are three basic morphological characteristics that must be taken into consideration: the size (Table 1), the germinal apertures (Table 1) and the shape (Figure 3).

In addition to morphological variation, the pollen grains are also differentiated by their nutritional and phytochemical composition (Campos et al., 2008). The percentage of certain ingredients in the composition of pollen depends mainly on the botanical origin, the geographic region where it is produced, the climate and the processing conditions (Komosinska-vassev et al., 2015). Pollen is considered a beneficial hive product for human consumption due to the ingredients that compose it (Campos et al., 2008). Pollen is mainly composed of water (20-30%), carbohydrates (fructose, glucose sucrose and fibres; 13-55%), proteins (10-40%) and fats (1-13%), but also contain other minor components, such as minerals and vitamins (Campos et al., 2008). Adding to its high nutritional value, many studies have shown that pollen also contains bioactive compounds and antioxidants such as carotenoids and polyphenols (phenolic acids and flavonoids) (Campos et al., 2008; Li et al., 2018). Moreover, in vitro studies evidenced that pollen presents very interesting therapeutic properties such as anti-inflammatory, antimicrobial, anticancer, antimutagenic and immunomodulatory properties (Pascoal et al., 2014).

Table 1: Different sizes and germinal apertures of pollen grains

Morphological characteristics		
Sizes	Very small	<10µm 10-24µm
	Small	25-49 µm
	Medium	50-99µm
	Large	
	Very large	100-200 µm
	Gigantic	>200 µm
Germinal apertures	Porate	Possess only pores
	Colpate	Possess only furrows
	Colporate	Possess pores and furrows

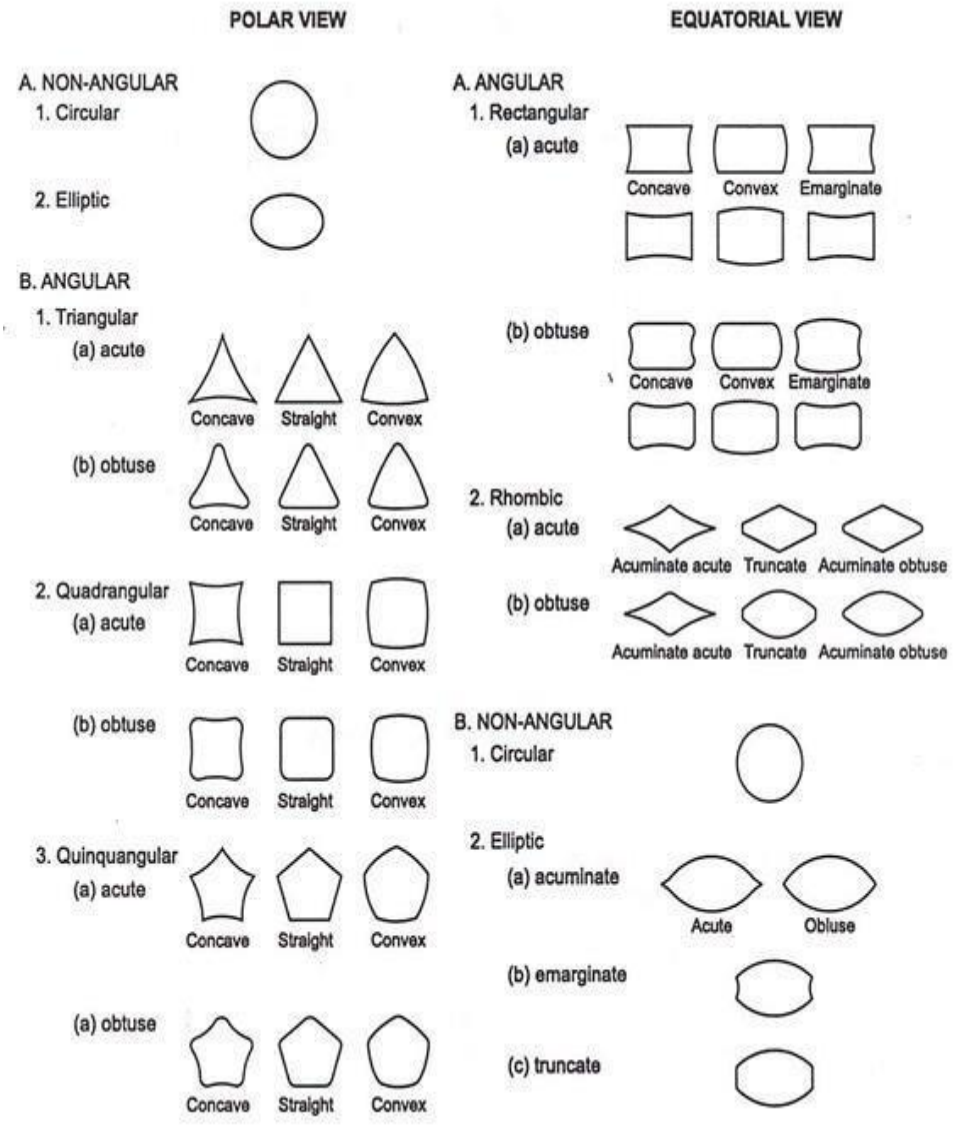


Figure 3: Shapes of pollen grains in polar and equatorial view (www.biologydiscussion.com).

4. Palynology

Palynology is the science that studies the pollen grains and spores (Erdtman, 1963). The identification of pollen collected by honey bees is an essential tool both in studies comprising pollinator foraging behaviour as well as for the authentication of bee hive products. In particular, the branch of palynology that studies the pollen contained in honey is designated as melissopalynology. Pollen analysis provides a fingerprint of the plants

visited by honey bees allowing determining the botanical origin of honey and indirectly its geographical traceability (Marquele-Oliveira et al., 2017). In addition to determination of botanical origin of honey, melissopalynological analyses allow the reliable estimation of the relative abundances of the different pollen taxa (quantification) required for honey labelling as monofloral or multiforal. While melissopalynology is the most common application of pollen analyses, as referred, botanical origin of mixed pollen samples can also be required for studies of plant-insect interactions, honey bee nutritional biology, pollinator foraging behaviour, among other purposes.

Palynological analysis by light microscopy is the most commonly used method for pollen identification having the advantage of providing reliable estimates of relative abundance of pollen taxa in a mixed pollen sample. However, this method also has a number of drawbacks, namely: it is time-consuming, it requires expert knowledge, it provides low taxonomic resolution for many plant taxa, as for several plant species it allows identification only at the family level, and it frequently fails in detecting rarer taxa (Bell et al., 2016).

With all these limitations, the development of new reliable techniques for pollen analysis becomes a necessity (Richardson et al., 2015). The application of DNA metabarcoding using next-generation sequencing (NGS) to pollen analysis presents a promising efficient alternative to the microscopic analysis.

5. Genomic DNA extraction from mixed pollen samples

Isolation of genomic DNA is an essential procedure for the identification of plants species in a mixed pollen sample by means of molecular biology approaches. Particularly, for DNA metabarcoding, it is of utmost importance to select an extraction protocol that yields high-quality DNA for amplification (Bell et al., 2016). In the case of mixed pollen samples, before starting the extraction of the genomic DNA, generally a sample preparation step is necessary to obtain a representative sampling by ensuring optimal sample mixing. This can be achieved by manual stirring followed by the random collection of subsamples for analysis. To extract DNA from pollen samples, a key step concerns the destruction of the very hard external layer, called the exine (Lalhmangaihi et al., 2014). For exine disruption, different methods are reported in the literature including homogenization using bead-beating or tissue lyser devices, either with or

without proteinase K, and pestle-based pulverization facilitated by liquid nitrogen deep freezing (Bell et al., 2016). According to Engel et al. (2012) the pectin-degrading enzymes from the honey bees gut microbiome, that enable the digestion of pollen, could potentially be used for DNA extraction of pollen, although this would require further studies to assess its effectiveness and possible implementation (Bell et al., 2016). After exine disruption, genomic DNA can be extracted either by using commercial kits (Table 2) or classical extraction protocols such as phenol-chloroform and cetyltrimethylammonium bromide (CTAB) methods. Among such approaches, commercial DNA extraction kits are the most frequently used.

Extraction of high-quality DNA from plant tissues rich in polysaccharides and polyphenols, such as pollen, is reported as being a fundamental step because these substances can affect either the quality and or quantity of nuclei acids isolated, but above all they may inhibit the *Taq* polymerase enzyme (Japelaghi et al., 2011; Rezadoost et al., 2016). To allow for a better quality of the extracted DNA, polyvinylpolypyrrolidone (PVP) can be used to adsorb polyphenolic compounds through formation of hydrogen bonds (Abdel-Latif et al., 2017).

A further consideration when extracting DNA from pollen is the contamination from external sources. Since pollen contains a significant amount of water and sugar, it presents a suitable environment for the growth of more than 15 genera of moulds and yeast, as previously reported (Estevinho et al., 2012; Petrovic et al., 2014; Kačániová et al., 2011; Barbosa et al., 2018). In fact, this contamination can cause problems during DNA extraction as the total extract will be composed of a mixture of different genetic material originating not only from plants but also from fungi. This will lead to erroneous quantification of the DNA originating from pollen and will consequently interfere with downstream NGS applications and pollen identification. Therefore, the storing conditions of pollen grains after being collected from pollen traps as well as during transportation to the laboratory facilities should also be taken into consideration. To avoid the mentioned problems, it is necessary to use pollen grains stored under conditions that inhibit the growth of any organism.

Table 2 : DNA extraction from mixed pollen samples using commercial kits.

Pollen sample	Homogenization/exine disruption	Commercial kit	Reference
0.01 g of fresh pollen	Freezing the mixture of pollen in liquid nitrogen, and grinding it into a fine powder	Plant DNeasy Isolation	Galimberti et al., 2014
0.003 g of fresh pollen	Tissue Lyser LT (Qiagen)	*NucleoSpin Food kit* (Macherey Nagel)	Keller et al., 2015 Sickel et al., 2015
0.05 g of dried pollen	Bead-beater pulverization with Mini-BeadBeater-1 (BioSpec Products)	*DNeasy Plant mini kit* (Qiagen)	Richardson et al., 2015
Not referred	Freezing the mixture of pollen in liquid nitrogen, followed by high-energy agitation with beads (RetschMM200 mixer mill)	*Nucleomag kit* (Macherey–Nagel) and *DNeasy Plant mini kit* (Qiagen)	Leontidou et al., 2018
Not referred	Mini-BeadBeater-96 (BioSpec Products)	NucleoSpin Food kit (Macherey Nagel)	Bell et al., 2017
Not referred	TissueLyser II (Qiagen) with tungsten carbide beads	DNeasy plant mini kit (Qiagen)	Lucas et al., 2018

6. Barcode genetic markers

Barcode markers are considered as fingerprint of species. They are used in taxonomy and ecological evolutionary research by facilitating species identification. The choice of markers used for amplification are of great importance for any study. For animals, the mitochondrial cytochrome c oxidase I gene (COI) is the DNA barcoding marker of choice (Hebert et al., 2003) .Its usefulness lies in the slower mutation rate when compared to other protein coding mitochondrial genes and has little within-species diversity. However, the use of the COI sequence is not appropriate in plants because of slower rate of cytochrome c oxidase I gene evolution in plants than in animals.

As for animals, in plants, the major requirements of a successful barcode marker isa high interspecific with low intra-specific variability. According to the Consortium forthe Barcode of Life (CBOL) Plant Working Group (2009), an ideal DNA barcode should be recoverable regularly with a single pair of primers, provide a bidirectional sequencing, and enable most species to be distinguished. Among the different loci, seven regions of the chloroplast genome (*rbcL, matK, rpoB, rpoC1, atpF–atpH, trnH-psb* and *psbK–psbI*) have been proposed for evaluation by CBOL Plant-Working Group (Hollingsworth et al., 2009), either used separately or in combination.

Based on criteria of universality, sequence quality, and levels of species discrimination every single locus has been evaluated separately, with results as follows:
(i) success of *rpoC1* and *rpoB* in terms of universality and/or sequence quality, but both had low discriminatory power; (ii) *atpF–atpH* presents high-quality bidirectional sequences, however it cannot be routinely sequenced across the land plants because of its low universality; (iii) *psbK–psbI* showed 68% to 69% species discrimination among 397 samples, but had the lowest sequencing quality (Hollingsworth et al., 2009). Taking into consideration all these criteria, *rpoC1, rpoB, atpF–atpH,* and *psbK–psbI* cannot be considered as ideal barcodes (Hollingsworth et al., 2009). In contrast, *trnH–psbA, rbcL*, and *matK* fits the norms that are highly desirable in a plant DNA barcoding system, although none of them fit the ideal DNA barcode marker perfectly. Therefore, the search for a universal barcode suitable for plant species has been pursued by several researchers including other non-chloroplast genome regions, such as nuclear regions. However, no single locus has been shown to be adequate for all cases, and thereby a combination of loci is frequently necessary (Techen et al., 2014). Nuclear fragments (e.g. ribosomal *ITS 2* region) as well as regions of chloroplast genome, with fast rates of evolution, have become suitable candidate DNA markers in plants (Hollingsworth et al., 2009; Li et al., 2011).

Table 3 shows different DNA barcodes described in the literature for species identification in studies of mixed pollen samples.

In contrast to other plant barcodes, the small size of the *ITS2* amplicon fragment (163-311 bp) is compatible with HTS, allowing sufficient overlap for paired-end merging. Furthermore, this marker has shown high discriminative capabilities at the genus and species level (Chen et al., 2010; Keller et al., 2014; Richardson et al., 2015), with 92.7% successful identifications at the species level in 6600 samples belonging to 4800 species

(Chen et al., 2010) and revealed to be superior to traditional microscopic identification for qualitative analysis (Keller et al., 2014; Richardson et al., 2015). However, the potential of *ITS2* for fungal co-amplification may be considered as a limitation of choice as a barcode (Cheng et al., 2016). While fungal co-amplification can lead to sequencing failure when using Sanger sequencing, because this method can only be used to sequence and identify a DNA extract from one species, the target DNA, which will be copied many times, will not really match plant DNA. Nevertheless, using HTS DNA metabarcoding, fungal contamination will be sequenced alongside the taxa of plants (Cheng et al., 2016). This will not prevent sequencing and identification of plant species, but the number of reads needed per sample will be increased, therefore the number of samples that can be analysed will be limited (Bell et al., 2016). As a proposed solution to overcome this limitation, newly designed primers with only plant-specific amplification, are proposed as an alternative to avoid fungal co-amplification. The efficacy of the new designed *ITS2* primers was proved by Cheng et al. (2016) while the new primer pairs gave PCR improvements up to 30% compared with common-used ones.

Table 3 : Summary of information available on plant DNA barcoding markers used in pollen DNA barcoding studies.

Locus	Location in the cell	Characteristic	Length (bp)	References
rbcL	Chloroplast	Coding gene	702–883	Bruni et al. 2015; Hawkins et al. 2015; Richardson et al. 2015
matK	Chloroplast	Coding gene	656–861	Richardson et al., 2015
trnH-psbA	Chloroplast	Non coding gene	103–1025	Bruni et al. 2015 ; Galimberti et al. 2014
ITS 2	Nucleus	Non coding gene	163–311	Keller et al. 2015 ; Richardson et al. 2015 ; Sickel et al. 2015

7. DNA metabarcoding high throughput sequencing (HTS)

DNA barcoding using traditional Sanger sequencing has been proposed as an alternative approach for identifying pollen (Bell et al., 2016). However, this technique is of little utility for mixed pollen identification. It requires isolation and sequencing of

individual pollen grains from the mixtures or to use cloning techniques (Hawkins et al., 2015; Keller et al., 2015), which are labour-intensive and error-prone tasks. Promising, in terms of addressing the issue of mixed-species identification and avoiding this hindrance, DNA metabarcoding has demonstrated the potential as a suitable alternative to both conventional pollen identification via light microscopy and barcoding Sanger-based sequencing (Bell et al., 2017).

DNA metabarcoding is a method that consists of identifying all the species found in an environmental sample. It allows the determination of organisms at different taxonomic levels and comparison of the composition of taxa among samples, without any need of a prior step of specimen sorting. It combines two technologies: DNA barcoding and high-throughput DNA sequencing (HTS) (Sickel et al., 2015).The identification of the species composition by DNA metabarcoding is ensured by a set of processes starting with bulk DNA extraction followed by DNA amplification using PCR, in order to raise the quantity of DNA for downstream analyses. Universal PCR primers are used to mass-amplify DNA barcodes from mass collections of organisms or from environmental DNA. The PCR products are subject to HTS and the result is a wealth of DNA sequences, which allows a direct assessment of the species that exist in the sample (Bell et al., 2016).

Among other approaches, DNA metabarcoding has been suggested as an effective methodology for species identification in mixed pollen samples (Figure 4). According to Bell et al. (2016), four components are needed to achieve a successful DNA metabarcoding pollen analysis: an extraction protocol that ensures high-quality DNA template for PCR-amplification; a set of genetic markers that can be successfully amplified (e.g. *ITS2*), a database containing reference sequences of the genetic markers for the majority of seed plant species, and a HTS method and bioinformatics pipeline that allows the simultaneous identification of plant species from a mixture of pollen.

NGS has overcome the limitations of classical DNA sequencing methods and has found usage in a wide range of molecular biology applications. However, several technical difficulties, despite being improved from one generation to another, emerged with these technologies (Sickel et al., 2015). Every sequencing generation and platform, which is based on different methodological approaches, has advantages and disadvantages. These will determine the suitability for certain applications. Figure 4 presents the different methods used for mixed pollen identification.

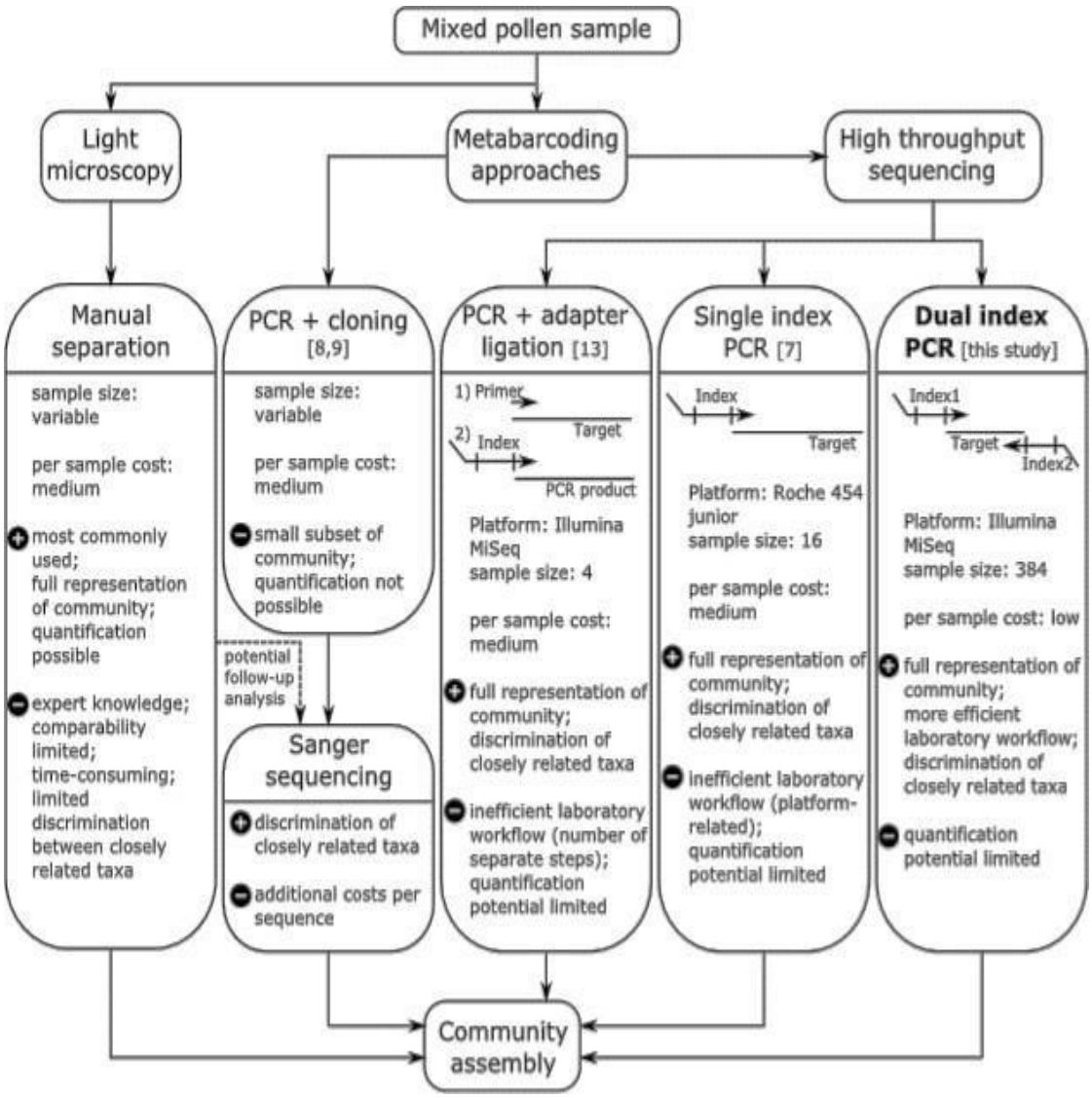


Figure 4: Comparison of different approaches for plant species identification in mixed pollen sample (Source: Sickel et al., 2015)

II. Materials and methods

1- Sampling

A total of 30 mixed pollen samples were collected from pollen traps in 7 countries across Europe: Austria, Denmark, France, Greece, Italy, Latvia and Portugal (Figure 5, Table 4). Most samples were shipped to CIMO's laboratory between November and February of 2019.

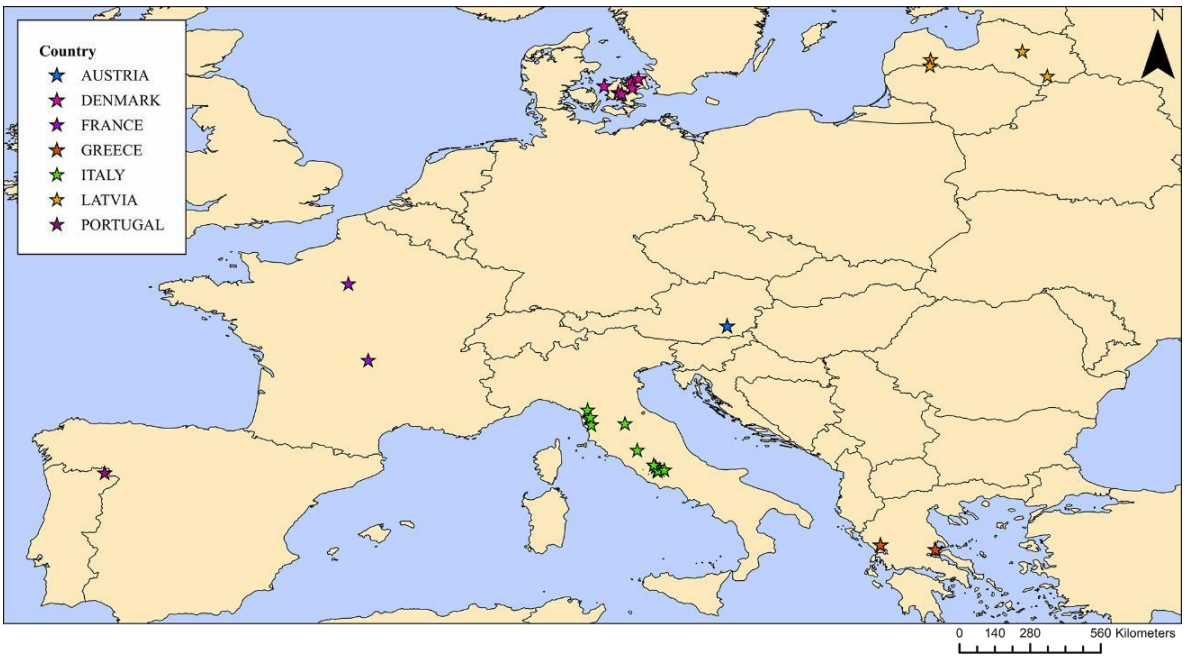


Figure 5: Map showing the geographic sites where the mixed pollen samples were collected.

Table 4: Sample size per country and sample IDs.

Country	Sample size	Sample ID
Austria	2	A37
France	2	F33, F34
Greece	2	Gb1, G36
Portugal	2	P31, P41
Latvia	5	L23, L26, L28, L29
Denmark	7	D16, D17, D19, D20 D21, D22
Italy	13	I2, I3, I4, I5, I6, I7, I8, I9 I10, I11, I12, I13, I15
Total	30	

2- Homogenization of mixed pollen samples

The 30 mixed pollen samples were homogenized prior to DNA extraction. To that end, 2 g of each of the 30 mixed pollen samples were weighted placed into a beaker and 10 mL of distilled water were added to cover the sample for steeping. The solution was mixed using a magnetic stirrer at a moderate speed for approximately 10 minutes or until all pollen pellets were broken down and the solution was homogenised. While the stirrer was in motion, sub-samples of 1 mL were taken, placed into a 1.5 mL Eppendorf, centrifuged at 10,000 x*g* for 3 minutes and the supernatant discarded. Then, the sample was transferred to a 2.0 mL screwtop microtube containing an in-house bead mix recommended by Keith Browne from NUI Galway University, Ireland. For the comparison of DNA extraction methods and evaluation of different storage methods, the bead mix was composed of 80 mg acid washed sand and zirconia beads of four different sizes, namely 38 mg of 200 µm, 85 mg of 400 µm, 97 mg of 800 µm and 2 beads of 3 mm. For metabarcoding identification the same mix was used but the 80 mg of washed sand was replaced by 80 mg of 100 µm zirconia beads.

3- DNA extraction: a comparison of methods

3.1- DNA extraction methods

The DNA of the 30 homogenized mixed pollen samples was extracted using four different methods, namely: NucleoSpin, GF-1 Plant, HigherPurity, and CTAB-PVP.

3.1.1- NucleoSpin method

The NucleoSpin method was based on the use of the commercial kit NucleoSpin® Food (Macherey–Nagel, Düren, Germany) and performed according to the manufacturer's instructions with some minor modifications. A volume of 550 µL of CF lysis Buffer preheated to 65°C was added to the 2.0 mL screwtop microtubes containing the pre-treated homogenised mixed pollen samples. The tubes were placed in a PRECELLYS® bead mill homogenizer equipment set at 6200 rpm for 5 seconds repeated 3 times. Then, 10 µL of Proteinase K solution (20 mg mL^{-1}) was added and vortex for 5 seconds. The samples were incubated at 65°C for 30 minutes and then centrifuged for 10 minutes at 10,000 x *g* to eliminate pellet contaminants and cell debris. An optional step was suggested by the manufacturer of adding RNase A (20 mg mL^{-1}) if RNA-free DNA is crucial for downstream applications (this protocol was performed with and without this additional step and the

results were compared). The supernatant was transferred into a microcentrifuge tube and added with one volume of Buffer C4 and one volume of ethanol (e.g., take 300 µL of the sample and add 300 µL of Buffer C4 and 300 µL of ethanol). The mixture was vortexed for 30 seconds and subsequently 700 µL were placed in a column containing a dry silica membrane filter, centrifuged for 1 minute for 11,000 x *g* and the filtered solution was discarded. The columns were washed three times: the first wash with 400 µL of Buffer CQW, the second and third washes with 700 µL and 200 µL of Buffer C5, respectively, followed by 1 minute centrifugation at 11,000 x *g* after the first and second washings and a centrifugation for 2 minutes at 10,000 x *g* after the final one in order to remove Buffer C5 completely. The DNA of the mixed pollen was eluted from the column by adding 100 µL of Buffer CE pre-heated at 70 °C, followed by a 5 minute incubation period at room temperature (18-25 °C), and then centrifuged for 1 minute at 11,000 x *g*.

3.1.2- HigherPurity method

The HigherPurity method was based on the use of the commercial kit HigherPurity™ Plant DNA Purification (Canvax Biotech, Spain) following the manufacturer's instructions. The pre-treated homogenised mixed pollen samples were transferred to a 1.5 mL microcentrifuge tube. Each sample was added with 400 µL of BL1A extraction Buffer and 20 µL of RNase A (10 mg mL^{-1})**.** The contents were mixed by vortexing vigorously. After mixing, each tube was incubated at 65°C for 10 minutes. After incubation, 130 µL of BL2 Buffer were added to the samples, mixed by vortexing and incubated on ice for 5 minutes. Each sample was transferred to the column placed in a 2 mL tube and then centrifuged at 10,000 x *g* for 3 minutes. The clarified filtrate was carefully transferred to a new 1.5 mL microcentrifuge tube and a volume of 1.5 mL of BL3 Buffer was added to the clarified lysate and mixed vigorously by vortexing. Then 750 µL of the mix were transferred to the DNAprep Mini Spin column, placed in a 2 mL collection tube, centrifuged at 10,000 x *g* for 1 minute and the flow-through from the collection tube was discarded. Then, the DNAprep spin column was washed, first by adding 400 µL of Wash Buffer 1 and then 650 µL of Wash Buffer 2. In both cases, the columns were centrifuging at 10,000 x *g* for 30 seconds and the flow-through was discarded. Finally, the DNAprep column was placed into a new 1.5 mL microcentrifuge tube and a volume of 100 µL of Elution Buffer (preheated at 65ºC) was pippeted directly into the centre of the column. The samples were incubated for 3 minutes at room temperature (18-25 °C) and centrifuged at 10, 000 x *g* for 1 minute to elute the DNA.

3.1.3- *GF-1 Plant method*

The GF-1 Plant method was based on the use of the commercial kit GF-1 Plant DNA Extraction (Vivantis Technologies Sdn. Bhd., Malaysia) and performed according to the manufacturer's instructions with minor modifications. Each pre-treated homogenised mixed pollen sample was placed in a 1.5 mL tube and 280 µL of Buffer PL was added. The mixture was vortexed for 30 seconds to obtain a homogeneous solution and then a volume of 20 µL of Proteinase K (20 mg mL^{-1}) was added. This solution was mixed by inversion for a few minutes, incubated at 65°C for 2 hours in a shaking water bath and centrifuged at 16,000 x *g* for 5 minutes to precipitate any insoluble/undigested materials. The supernatant containing the DNA was transferred into a clean 1.5 mL microcentrifuge tube and 2 volumes of Buffer PB were added. The mixture was thoroughly homogenized by inverting the tube several times and then incubated for 10 minutes at 65°C. Each sample was added with 200 µL of absolute ethanol, mixed immediately and thoroughly, transferred into a column assembled in a clean collection tube and centrifuged at 10,000 x *g* for 1 minute. The supernatant was discarded and the DNA pellet was washed with 650 µL of Wash Buffer and centrifuged at 10,000 x *g* for 1 minute. The supernatant was discarded and DNA was eluted to a new tube using 100 µL of Elution Buffer.

3.1.4- CTAB-PVP method

The CTAB-based method was performed as described by Mafra et al. (2008) with minor modifications. A total of 1 mL of CTAB-PVP extraction buffer [1% polyvinylpyrrolidone (PVP-40); 20 mM EDTA; 100 mM TrisHCl; pH 7.5; 1.4 M NaCl; 2% w/v CTAB (cetyl-trimethylammonium bromide) and 20 mL of β-mercaptoethanol] was added to each pre-treated homogenised mixed pollen sample. The suspension was vortex stirred and then incubated for 3 hours at 65 °C. The suspension was centrifuged for 15 minutes at 17,000 x *g* (4 °C), the supernatant was transferred into a new tube and centrifuged again for 5 minutes (17,000 x *g*, 4 °C). Then, the supernatant was transferred into a new tube and added with 500 µL of chloroform, vortex stirred and centrifuged for 10 minutes at 12,000 x *g* (4 °C). The upper phase was transferred to a new tube, mixed with a double volume of a CTAB precipitation solution (5 g/L, 0.04 M NaCl) and incubated for 3 hours at room temperature. After centrifugation for 10 minutes at 12,000 x *g* (4 °C), the supernatant was discarded and the precipitate was dissolved in 350 µL of 1.2 M NaCl solution and extracted with 350 µL chloroform. The mixture was centrifuged for 10 minutes (12,000 x *g*, 4 °C) and the upper phase was mixed by inversion with 0.6 volume parts of isopropanol

at -20 °C. The mixture was centrifuged for 10 minutes (12,000 x *g*, 4 °C), the supernatant was discarded and the pellet was washed with 500 µL of ethanol solution (70%, v/v) at -20 °C. After centrifugation, the supernatant was carefully discarded, the pellet was dried and the DNA was eluted in 100 µL of TrisEDTA buffer (10 mM Tris, 1 mM EDTA).

3.2- DNA assessment

The quantity and quality (yield and purity) of the DNA isolates extracted using the aforementioned methods was evaluated by electrophoresis using a horizontal gel Mini-Sub® Cell (Bio-Rad) in a 1.0% agarose gel and by spectrophotometry. The agarose gel was visualized under UV light and a digital image was obtained using ChemiDoc™ XRS+ System with Image Lab™ Software (Bio-Rad). The UV spectrophotometry was performed using a microplate spectrophotometer system (Biotek-Epoch 2) with a Take 3 micro-volume plate accessory and the nucleic acid quantification protocol with sample type defined for double-stranded DNA in the Gen5 data analysis software version 3.04 (BioTek Instruments). Absorbance readings were made at 260 nm (A260) and 280 nm (A280) in order to estimate DNA content and purity. All readings were made in duplicate. DNA purity is given by the ratio A_{260}/A_{280}, which preferably should fall between 1.8 and 2.0 (Sambrock et al., 1989), with lower values denoting presence of proteins, phenols and other contaminants and higher values denoting RNA contamination.

All the DNA samples were diluted to a concentration of 100 ng/µl for downstream polymerase chain reaction (PCR) applications.

3.3- PCR conditions

The primers used for PCR amplification of the DNA isolates are given in Table 5. Two different plant barcoding *ITS2* primer pairs were used. The first primer pair is a combination proposed by Sickel et al. (2015) of ITS-S2F (Chen et al., 2010) and ITS-4R (White et al., 1990). The second primer pair was designed and validated by Cheng et al. (2016).

All PCR reactions were performed in a T100™ Thermal Cycler (Bio-Rad) using different master mixes, reactions and temperature profiles, as detailed in Tables 6.a, b, c, and d. Assessment of PCR products was performed in a 1 % agarose gel electrophoresis using the equipment described above.

Table 5 : Oligonucleotide primers and adaptors used in PCR.

Gene	Name	Sequence 5′ – 3′	Reference
ITS2	ITS-S2F	ATGCGATACTTGGTGTGAAT	(Chen et al., 2010)
	ITS4R	TCCTCCGCTTATTGATATGC	(White et al., 1990)
ITS2	ITS-u3	CAWCGATGAAGAACGYAGC	(Cheng et al., 2016)
	ITS-u4	RGTTTCTTTTCCTCCGCTTA	
Adaptors	F	TCGTCGGCAGCGTCAGATG TGTATAAGAGACAG	CIBIO (Research Centre in Biodiversity and Genetic Resources)
	R	GTCTCGTGGGCTCGGAGAT GTGTATAAGAGACAG	

3.3.1- QIAGEN ®Multiplex Master mix PCR Kit

The PCR reaction and the temperature profile for the *QIAGEN® Multiplex Master mix PCR* kit (Table 6) was carried out as suggested by the QIAGEN Multiplex PCR handbook, with the optimal annealing temperature as suggested by Sickel et al. (2015) for the primers ITS-S2F (Chen et al., 2010) and ITS-4R (White et al., 1990).

3.3.2- GoTaq® Flexi PCR kit

The PCR reaction and the temperature profile for the *Promega® GoTaq Flexi* kit (Table 7) were performed according to manufacturer's instructions with an optimization of the annealing temperature as suggested by Sickel et al. (2015) for the primers ITS-S2F (Chen et al., 2010) and ITS-4R (White et al., 1990).

3.3.3- Q5®High-Fidelity PCR Kit

The PCR reaction and the temperature profile for the *Q5®High-Fidelity PCR* kit (Tables 8 and 9) were performed according to manufacturer's instructions with minor modifications of the annealing temperature to have an optimal Tm for the two primer pairs.The four DNA isolation methods (section 3.1) were compared for DNA yield and purity, as described in section 3.2, and the PCR protocols detailed in sections 3.3.1 and 3.3.2 were performed to further evaluate the extracted DNA.

Table 6 : PCR conditions used for the amplification of the ITS2 region using the primers ITS-S2F and ITS4R and the *QIAGEN® Multiplex Master mix PCR kit.*

Reaction Mix (10 µL)		
1x Qiagen Master Mix		5 µL
0.2 µM Primer Forward		0.5 µL
0.2 µM Primer Reverse		0.5 µL
H_2O		3 µL
Genomic DNA		1 µL
Amplification program		
Initial denaturation	95°C, 15 minutes	
Denaturation	94°C, 30 seconds	35x
Annealing	52°C, 40 seconds	
Extension	70°C, 60 seconds	
Final extension	60°C, 30 minutes	

Table 7: PCR conditions used for the amplification of the ITS2 region using the primers ITS-S2F and ITS-4R and *the Promega® GoTaq Flexi kit.*

Reaction Mix (25 µL)		
5x GoTaq Flexi Colourless Buffer without $MgCl_2$		5 µL
1.5 mM $MgCl_2$		2.5 µL
1.25 U GoTaq Flexi DNA Polymerase		0.125 µL
0.2 mM dNTPs		2.5 µL
0.2 µM Forward/ Reverse Primers		2.5 µL
H_2O		10 µL
Genomic DNA		1 µL
Amplification Program		
Initial denaturation	94°C, 2 minutes	
Denaturation	94°C, 10 seconds	30x
Annealing	52°C, 20 seconds	
Extension	72°C, 60 seconds	
Final extension	60°C, 5 minutes	

Table 8 : PCR conditions used for the amplification of the ITS2 region using the primers ITS-S2F and ITS-4R and the *Q5®High-Fidelity PCR kit.*

Plate1:Reaction Mix (10 µL)		
1xQ5 Master Mix		5 µL
0.5 µM Primer Forward		0.5 µL
0.5 µM Primer Reverse		0.5 µL
H_2O		3 µL
Genomic DNA		1 µL
Amplification Program		
Initial denaturation	98°C , 3 minutes	
Denaturation	98°C, 10 seconds	35x
Annealing	52°C, 30 seconds	
Extension	72°C, 40 seconds	
Final extension	72°C, 2 minutes	

Table 9 : PCR conditions used for the amplification of the ITS2 region using the primer pair ITS-u3/ITS-u4 (Cheng et al., 2016) and the *Q5®High-Fidelity PCR kit.*

Reaction Mix (10 µL)		
1xQ5 Master Mix		5 µL
0.2 µM Primer Forward		0.5 µL
0.2 µM Primer Reverse		0.5 µL
H_2O		3 µL
Genomic DNA		1 µL
Amplification Program		
Initial denaturation	98°C , 3 minutes	
Denaturation	98°C, 10 seconds	35x
Annealing	55°C, 30 seconds	
Extension	72°C, 40 seconds	
Final extension extension	72°C, 2 minutes	

4- Comparison of different pollen storage and transportation methods

4.1- Comparison between dry and wet pollen

Genomic DNA was extracted from pollen collected from pollen traps located in an apiary of Greece (Figure 5, Table 4). The Greek sample was split into 2 sub-samples: one was dried for 2 days at 25° C and the other one was stored fresh at -20° C. The 2 sub-samples were shipped to CIMO for DNA extraction from 5 replicates each, using the NucleoSpin method, as described in section 3.1.1. The quality (yield and purity) of the DNA extracts was evaluated by UV spectrophotometry and gel electrophoresis, as described in section 3.2. For further assessment of the extracted DNA, PCR was performed following the conditions detailed in Table 7 and 8.

4.2- Comparison of different pollen storage

In order to evaluate the impact of long-term pollen storage (at the laboratories of the international INSIGNIA consortium) and transportation (shipping from the INSIGNIA participating countries to CIMO) on the DNA quantity and quality, one sample of pollen from Portugal (Figure 5, Table 4) was divided into four sub-samples of 5 g each and submitted to four different storage conditions, namely: drying with silica for 11 days, oven drying at 25°C for two days, placed in absolute ethanol and freezing at -20 °C. Each storage treatment was tested using 5 replicates. Each sub-sample was submitted to DNA extraction using the NucleoSpin method, as described in section 3.1.1. The quality and yield of the DNA extracts was evaluated by UV spectrophotometry and gel electrophoresis, as described in section 3.2. For further assessment of the extracted DNA, PCR was performed following the conditions detailed in Table 7.

5. DNA metabarcoding high throughput sequencing (HTS) of ITS2

5.1-DNA extraction, PCR amplification, library preparation and sequencing

Botanical identification by DNA metabarcoding HTS of ITS2 was performed for the 30 samples covering a wide geographical range (Figure 5, Table 4). The DNA of these samples was extracted using the *NucleoSpin* method (section 3.1.1). The DNA yield and purity was assessed by electrophoresis and UV spectrophotometry (section 3.2). DNA amplification was performed using two different *ITS2* primer pairs modified to contain adaptors (Table 5) for sequencing in the Illumina platform. The PCR conditions were designed to accommodate the use of Q5® high-fidelity PCR kit, as described in section

3.3.2. Two 96-well PCR plates containing the 30 samples (3 replicates of each sample) and negative controls were prepared for HTS. Plate 1 contained the DNA samples amplified with the ITS2 primer pair ITS-S2F/ITS4R and Plate 2 contained the samples amplified with ITS-u3/ITS-u4 primer pair. PCR products were sent to the laboratory of CIBIO (https://cibio.up.pt/), University of Porto, for library preparation using an in-house protocol and for high-throughput sequencing in a single flow cell on the Illumina MiSeq platform using 2×250 cycles v2 Illumina chemistry.

5.2- Bioinformatics pipeline and data analysis

The bioinformatics pipeline and the European ITS2 reference database used for the analysis of the MiSeq reads were provided by Dr. Alexander Keller from University of Wurzburg, Germany. The pipeline was made available by Sickel et al. (2015) in https://github.com/iimog/meta-barcoding-dual-indexing.) and recently modified by Dr. Alexander Keller. The pipeline includes two classifications: the first one is direct and the second one is hierarchical. The main purpose of the direct classification was to classify all the sequence reads at the species level, against the European ITS2 reference database using a similarity threshold of 99%. The second classification was used to classify hierarchically to the genus, family, or order levels all the sequence reads that failed direct classification at the species level. The classification results obtained for the two PCR plates amplified using the two *ITS2* primer pairs were compared.

IV. Results and discussion

1. Comparison between dry and wet pollen

DNA was extracted from dry and wet pollen of a sample collected in Greece using the NucleoSpin method. The quality of the isolated DNA was evaluated on a 1% agarose gel electrophoresis and by UV spectrophotometry (Figure 8 and Table 7). The results of the agarose gel electrophoresis show that DNA was successfully isolated for the 10 pollen replicates, as revealed by the sharp high-weight molecular bands (>1 kb) and the smear, which indicates the presence of degraded DNA (Figure 6). For the DNA extracts obtained from the 5 wet pollen replicates, a RNA contamination was observed, especially for replicate W2 and to a lesser extent for W1, W4 and W5.

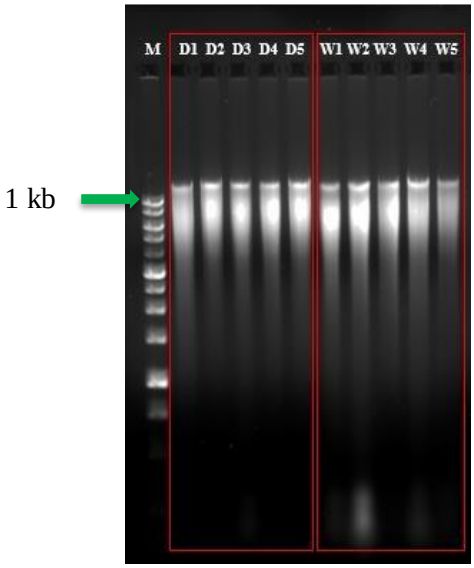


Figure 6: Agarose gel electrophoresis of DNA extracted from dry and wet mixed pollen sub-samples. DNA ladder (M).

The results of UV spectrophotometry are shown in Table 10. The absorbance ratio 260/280 was used to assess the quality of the DNA extracts, with an absorbance 260/280 ratio of ~1.8 denoting a higher DNA purity**.** For the dry pollen, all replicates produced good DNA quality since the absorbance ratio 260/280 was closer to 1.9±0.1. The DNA purity obtained with the wet replicates was lower as the A260/280 values were slightly higher (2.0±0.1), which can be explained by the presence of a higher content of RNA consistent with the results of the electrophoresis (Figure 6). The concentration mean values of the wet pollen (385.1±294.2 ng/µL) are higher than those of the dry pollen (144±56.3 ng/µL), which

can be explained by the higher amounts of RNA in the wet extracts. As will be latter detailed, RNA contamination can be prevented by adding an RNase treatment step during the DNA extraction, which was done for the 30 samples that were sequenced in the Illumina platform.

Table 10 : Results of DNA yield and purity obtained for the dry (D) and wet (W) mixed pollen samples.

	DNA extract ID	Purity 260/280	Concentration ng/µL	CV (%)
Dry pollen	D1	1.9	88.7	1.5
	D2	1.9	124.5	11.4
	D3	1.9	238.7	1.8
	D4	1.9	140.8	1.3
	D5	1.9	127.3	0.4
Wet pollen	W1	2.0	232.8	1.7
	W2	2.0	878.1	1.3
	W3	1.9	176.3	0.8
	W4	2.0	436.7	0.5
	W5	2.0	201.6	1.4

CV - coefficient of variation.

Comparing the obtained results for DNA extracts, both methods are similar regarding the DNA quality. However, concerning the DNA yield, higher concentrations were obtained from wet pollen. Nevertheless, since some RNA contamination was visible, as mentioned, the absorbance values can possibly be higher due to this.

In addition, it is known that the high humidity of the wet pollen presents an ideal culture medium for microorganisms like bacteria and fungi (Nikolaieva et al., 2019; Mauriello et al., 2017). Therefore, the use of dried samples may prevent microbial growth and pathogen survival after the drying process (Mauriello et al., 2017). Thus, considering this aspect and that, in general, the extracts obtained from dry pollen presented a better purity with a reasonable DNA yield, it was decided to proceed with dry pollen in the next experiments.

DNA extracts obtained from wet and dry pollen were also used to assess any possible influence associated with the use of a different enzyme mix.

Figure 7 illustrates the results of the PCR amplifications of *ITS2* region with the

primer pair ITS-S2F and ITS4R (Table 5) and the PCR conditions using the *QIAGEN ®Multiplex Master mix PCR Kit* (Table 7;) and the *Promega GoTaq® Flexi PCR kit* (Table 8). Comparing the two obtained results using *QIAGEN ®Multiplex Master mix PCR Kit* and *Promega GoTaq® Flexi PCR kit*, the amplification showed stronger PCR products with the expected size of 450 bp (the size of the expected amplicon using primer pair ITS-S2F/ITS-4R) using the *QIAGEN ®Multiplex Master mix.* However, the appearance of other bands, especially that of >900 bp, denotes nonspecific amplifications, which is a problem for downstream HTS applications.

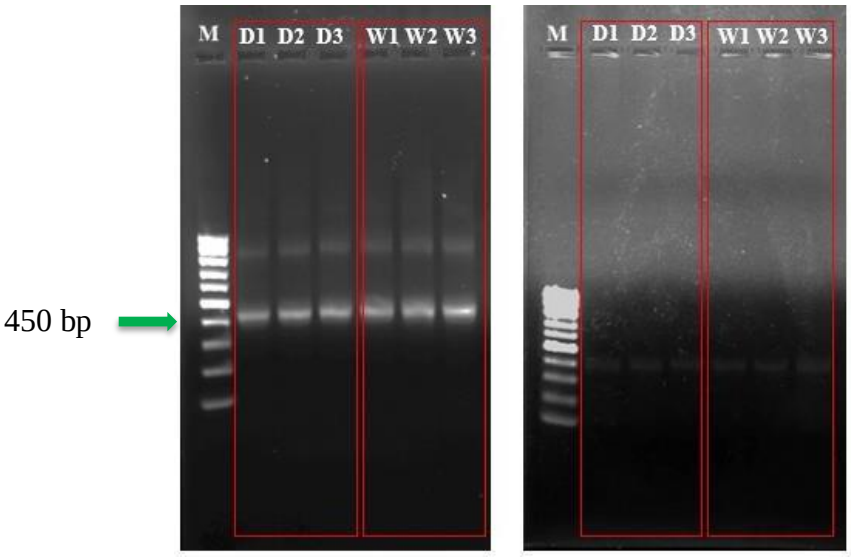


Figure 7: Agarose gel showing the PCR products amplified from DNA extracted from dry and wet mixed pollen DNA using the PCR conditions using the *QIAGEN ®Multiplex Master mix PCR Kit* (left) and the *Promega GoTaq® Flexi PCR kit* (right). DNA ladder (M).

2. Comparison of genomic DNA extraction methods

Based on the previous results, DNA was extracted only from dry pollen using the same homogenization step for all the samples in order to compare the four different DNA isolation methods: NucleoSpin, HigherPurity, GF-1 Plant, and CTAB-PVP. The results of agarose gel electrophoresis (Figure 8) show the presence of DNA only for extractions using the NucleoSpin and the CTAB-PVP methods. The HigherPurity and the GF-1 Plant methods did not produce any DNA, as far as determined by the sensitivity of the ChemiDoc™ XRS+ System. Comparing the gels obtained for the DNA extracted using the NucleoSpin and the CTAB-PVP, the former method was able to produce higher DNA yield and quality, as revealed by the presence of bands of high molecular weight (>1 kb).

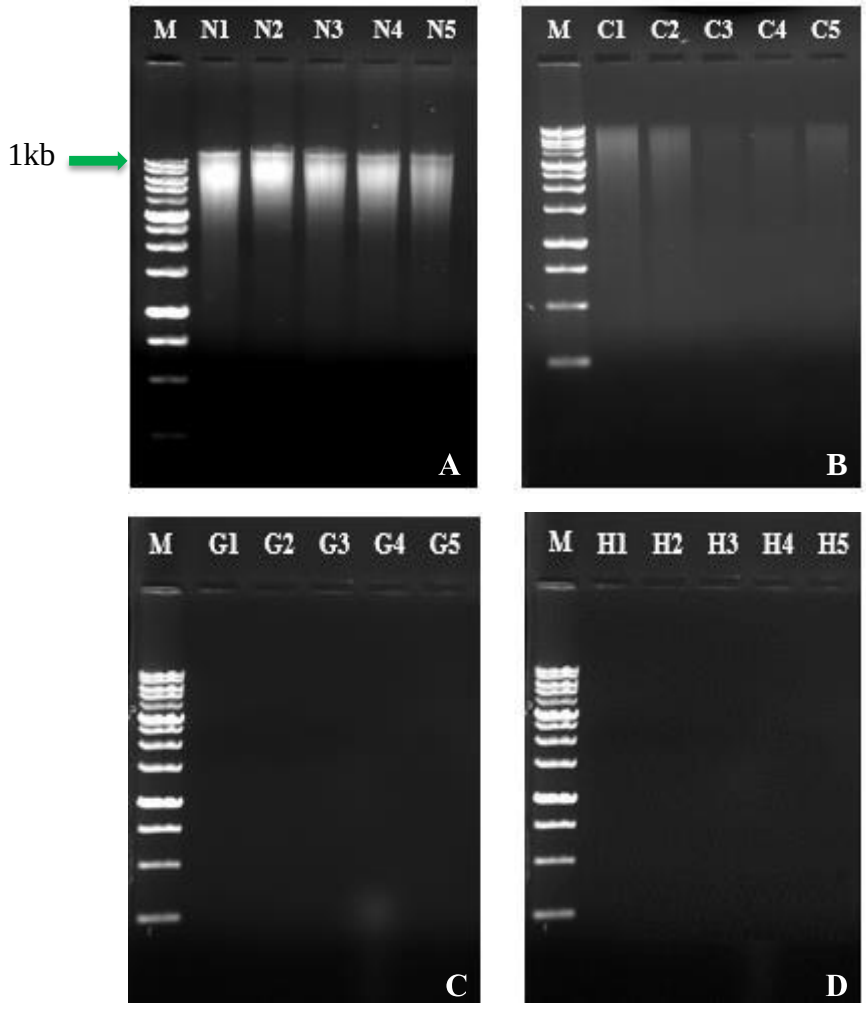


Figure 8: Electrophoresis results for the genomic DNA obtained by the four DNA extraction methods tested. A: NucleoSpin method; B: CTAB-PVP method; C: GF-1 Plant method; D: HigherPurity method. DNA ladder (M).

The results of the quantification with UV spectrometry were in good agreement with the electrophoresis analyses, confirming the highest DNA yields for all the samples extracted with the NucleoSpin method (144.0±56.3 ng/µL) followed by CTAB-PVP, which presented considerable lower values (28.2±17.7 ng/µL), (Table 8). The concentrations of extracted DNA using HigherPurity (3.5±2.0 ng/µL), and GF-1 Plant (7.2±1.7 ng/µL) methods in all the samples are negligible compared to the concentration of the extracts using the NucleoSpin and CTAB-PVP methods.

The DNA purity values, as determined by the 260/280 absorbance ratio, were 1.9±0.1 and 2.0±0.2 for the NucleoSpin and CTAB-PVP methods, respectively (Table 12). Therefore, the NucleoSpin method allowed obtaining extracts with higher purity. Similar results were previously reported by Bell et al. (2017), who considered the NucleoSpin Food kit as the best quality/yield method for DNA extraction from mixed pollen samples.

Table 11 : Results of DNA yields and purity of mixed pollen samples using the four DNA extraction methods.

DNA extraction method	Sample ID	Purity 260/280	Concentration ng/µL	CV (%)
NucleoSpin	N1	1.9	88.7	1.5
	N2	1.9	124.5	11.3
	N3	1.9	238.7	1.8
	N4	1.9	140.8	1.3
	N5	1.9	127.3	0.4
HigherPurity	H1	2	4.7	62.6
	H2	2.9	1.9	23.4
	H3	2	6.2	4.7
	H4	2.9	1.8	10.5
	H5	2.6	2.8	5.5
GF-1	G1	1.7	7.7	20.4
	G2	1.7	5.8	3.8
	G3	1.8	5.7	15.9
	G4	1.8	9.8	23.1
	G5	1.8	6.8	3.2
CTAB-PVP	C1	2	55.6	5.1
	C2	2	35.7	0.3
	C3	2.2	11.9	27.7
	C4	2.2	16.6	3.1
	C5	2	21.1	18.2

CV – coefficient of variation.

To further evaluate the efficacy of NucleoSpin and the CTAB-PVP methods, the 10 DNA extracts were amplified using the primer pairs ITS-S2F and ITS4R. The PCR products were assessed by electrophoresis. The gel image showed strong bands of the expected 450 bp size for all the samples (Figure 9), suggesting that all extracts had capacity for PCR amplification. Nevertheless, considering that the NucleoSpin method allowed obtaining extracts with higher yield and purity, it was consequently selected to be used in the next experiments.

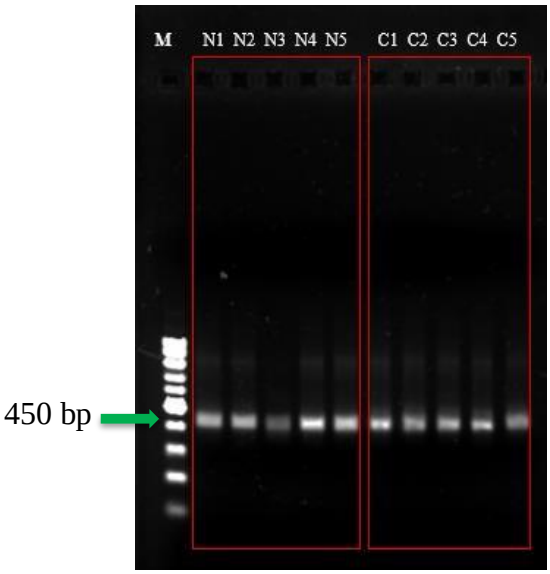


Figure 9: Agarose gel showing the PCR products amplified from DNA extracted from dry pollen with NucleoSpin (left) and CTAB-PVP (right) methods and PCR conditions of Table 7. DNA ladder (M).

3. Comparison of storage conditions

In order to evaluate the impact of pollen storage and transportation on the DNA quantity and quality, 20 sub-samples corresponding to 4 storage treatments (dried with silica, dried at 25°C for 2 days, placed in absolute ethanol, and frozen at -20 °C) x 5 replicates were assessed. The DNA of the 20 pollen sub-samples was extracted using the NucleoSpin method. The results of the agarose gel electrophoresis showed that the sub- samples stored in absolute ethanol evidenced higher DNA quality, as revealed by the presence of bands of high molecular weight (Figure 10). Nonetheless, this storage method, as well as the other three methods, produced degraded DNA, as revealed by the smear in the gel (Figure 10). A high RNA contamination was also visualized on the agarose gel.

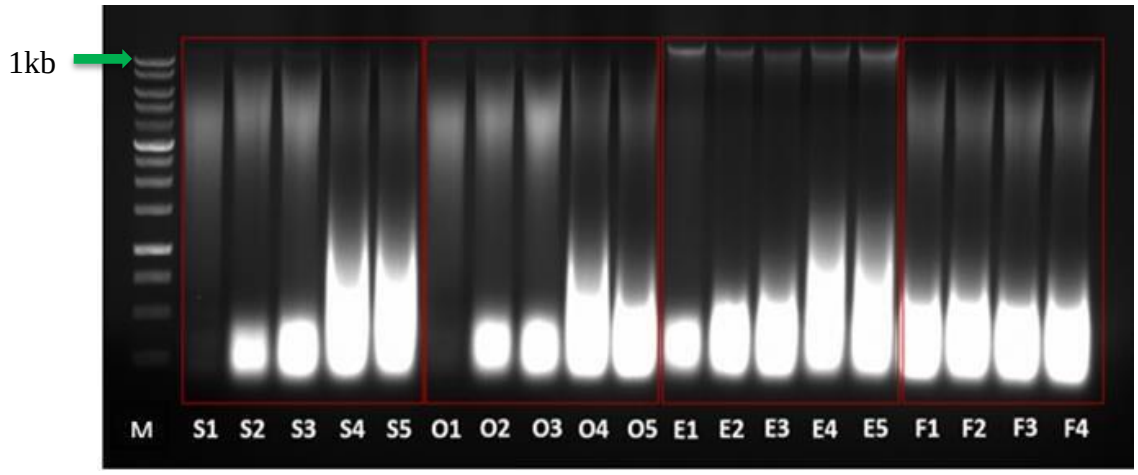


Figure 10 : Electrophoresis results for the NucleoSpin DNA extraction from pollen samples stored using four methods: dried with silica for 11 days (S), dried in the oven (O), placed in absolute ethanol (E) and frozen at -20 °C (F).DNA ladder (M).

The results of DNA yield and purity, obtained by UV spectrometry, of the mixed pollen sub-samples submitted to the four tested storage methods are presented in Table 10. The quantification results were in agreement with the electrophoresis analysis (Figure 10), which evidenced RNA contamination across the four storage methods. The presence of RNA explains the 260/280 absorbance ratios above 2.0 for most sub-samples and very high concentration values, which exceeded 3000 ng/µL in some cases (Table 13). As such, DNA yield of each sub-sample is unreliable, but based on the electrophoresis results only the samples stored in absolute ethanol produced DNA with lower degradation.

Altogether, these results suggest that pollen storage in absolute ethanol is superior to other methods concerning DNA degradation. Employment of ethanol for storing pollen has additional advantages, such as ease of use by beekeepers, low cost comparing to other products used in pollen storage such as liquid nitrogen, and protection from microorganisms' contamination, due to the antiseptic properties of ethanol. Many studies have suggested to combine absolute ethanol storage with cold for optimal preservation conditions (Hajibabaei et al., 2012).

However, the quality of the extracts can be optimized by adding an RNase step to eliminate RNA contamination from the extracts to obtain 260/280 absorbance ratios closer to the ideal 1.8 threshold. Two different RNase concentrations, 10 ng/µL and 20 ng/µL, and incubation times, 1 hour and 30 minutes, respectively, were compared for their efficiency in removing RNA from the extracts. The results, assessed using agarose gel electrophoresis, showed that both protocols were efficient as no traces of RNA were observed in the extracts after including the RNase treatment step in the NucleoSpin method (Figure 11).

Table 12: UV spectrometry results of DNA yield and purity of the mixed pollen sub-samples extracted with the four storage methods.

Storage method	Sample name	Purity 260/280	Concentration ng/µL	CV (%)
Dried with Silica	S1	1.9	176.3	0.6
	S2	2.1	852.7	1.2
	S3	2.1	1052.2	0.2
	S4	1.8	3435.4	0.7
	S5	1.8	3425.9	0.7
Dried at 25ºC	O1	1.9	156.4	0.1
	O2	2.1	1017.1	0
	O3	2.1	1113.2	0
	O4	1.7	3475.3	0.5
	O5	2.0	3217.9	0.6
Ethanol	E1	2.1	807.3	0
	E2	2.2	1696.8	0.8
	E3	2.1	2323.8	0.3
	E4	2.2	2189.8	1.1
	E5	2.2	2646.2	0
Frozen at -20ºC	F1	2.1	2747.6	0.1
	F2	2.1	2546.8	1.3
	F3	2.1	2273.8	0.6
	F4	2.1	2831.1	0.4
	F5	2.1	1142.6	0.3

CV - coefficient of variation.

The results of DNA yields and purity of the mixed pollen samples extracted with the NucleoSpin using the two RNase treatments are shown in Table 14. The purity values obtained for all the samples were ~1.7. The yield was slightly higher for the RNase treatment with 20 ng/µL for 30 minutes (65±13.9 ng/µL) than for 10 ng/µL for 1 hour (57.1±8.7 ng/µL). RNase with the concentration of 20 ng/µL shows good activity in a shorter time, which gives the advantage of gaining time. However, by using RNase with the concentration of 10 ng/µL there is an advantage of using a lower amount of the enzyme and therefore a lower cost.

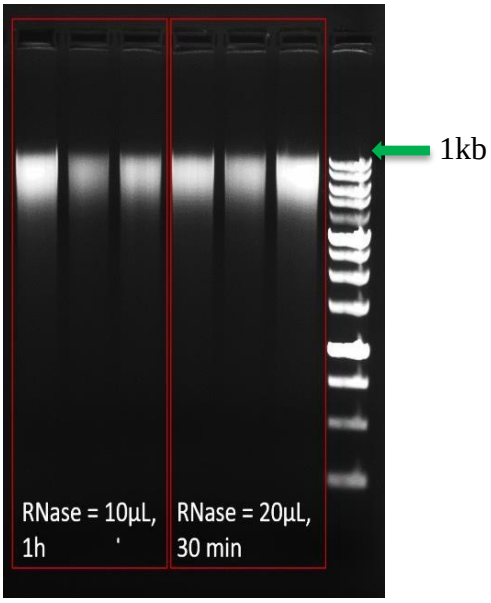


Figure 11: Electrophoresis results for the DNA extracts obtained by the NucleoSpin method with two RNase treatments: 10 ng/µL for1hour (left) and 20 ng/µL for 30 minutes (right).

Table 13: Results of DNA yields and purity of mixed pollen samples extracted with NucleoSpin method using two RNase treatments.

RNase treatment	Sample ID	Purity 260/280	Concentration ng/µL	CV (%)
10 µL, 1hour	F2.1	1.7	66.9	1
	F2.2	1.6	50.5	0.4
	F2.3	1.7	53.8	0.1
20 µL, 30 minutes	F2.4	1.7	55.5	1.6
	F2.5	1.7	58.5	1
	F2.6	1.7	80.9	0.4

CV - coefficient of variation.

In the extraction of DNA from the 30 samples of mixed pollen (metabarcoding experiment) using the Nucleospin method, the use of 20 ng/µL for 30 minutes has been adopted to save time.

4- Identification of plant taxa and relative abundances by HTS metabarcoding

The yield of the 30 DNA extracts of the mixed pollen samples collected across Europe (Figure 5) ranged between 11.8 and 273.7 ng/µL (Table 15). After diluting the DNA extracts to 10 ng/µL, the 30 samples were amplified using the two different *ITS2* primer pairs (Table 5). The agarose gel electrophoresis of the PCR products showed a successful amplification for virtually all the samples, with the expected size of 450 bp and the absence of non-specific amplifications (Figures 12, 13).

Table 14: Results of DNA yields of mixed pollen samples.

Country	Sample ID	Pollen (mg)	Mean (ng/µL)	CV (%)
Greece	Gb1	35	51.6	1.2
	G36	31	64.5	0.9
Italy	I2	27	44	1.4
	I3	25	94.1	0.6
	I4	81	10.3	5.9
	I5	38	15.4	3.9
	I6	35	10.3	1.2
	I7	79	151.6	0.9
	I8	55	109.0	0.4
	I9	46	134.3	0.6
	I10	47	22.5	5.9
	I11	43	11.8	3.9
	I12	31	31.7	1.4
	I13	72	37.1	1.6
	I15	34	66.6	0.9
Denmark	D16	32	48.8	1.8
	D17	35	61.9	0.7
	D19	39	273.7	2.7
	D20	39	48.1	0.6
	D21	42	52.2	0.5
	D22	40	32.3	2.7
Latvia	L23	34	94.6	5.2
	L26	43	140.1	0.4
	L28	97	26.0	1.1
	L29	37	137.6	0.3
Portugal	P31	34	58.4	1.2
France	F33	66	36.2	3.1
	F34	50	14.8	1.0
Austria	A37	76	19.5	1.2

CV - coefficient of variation.

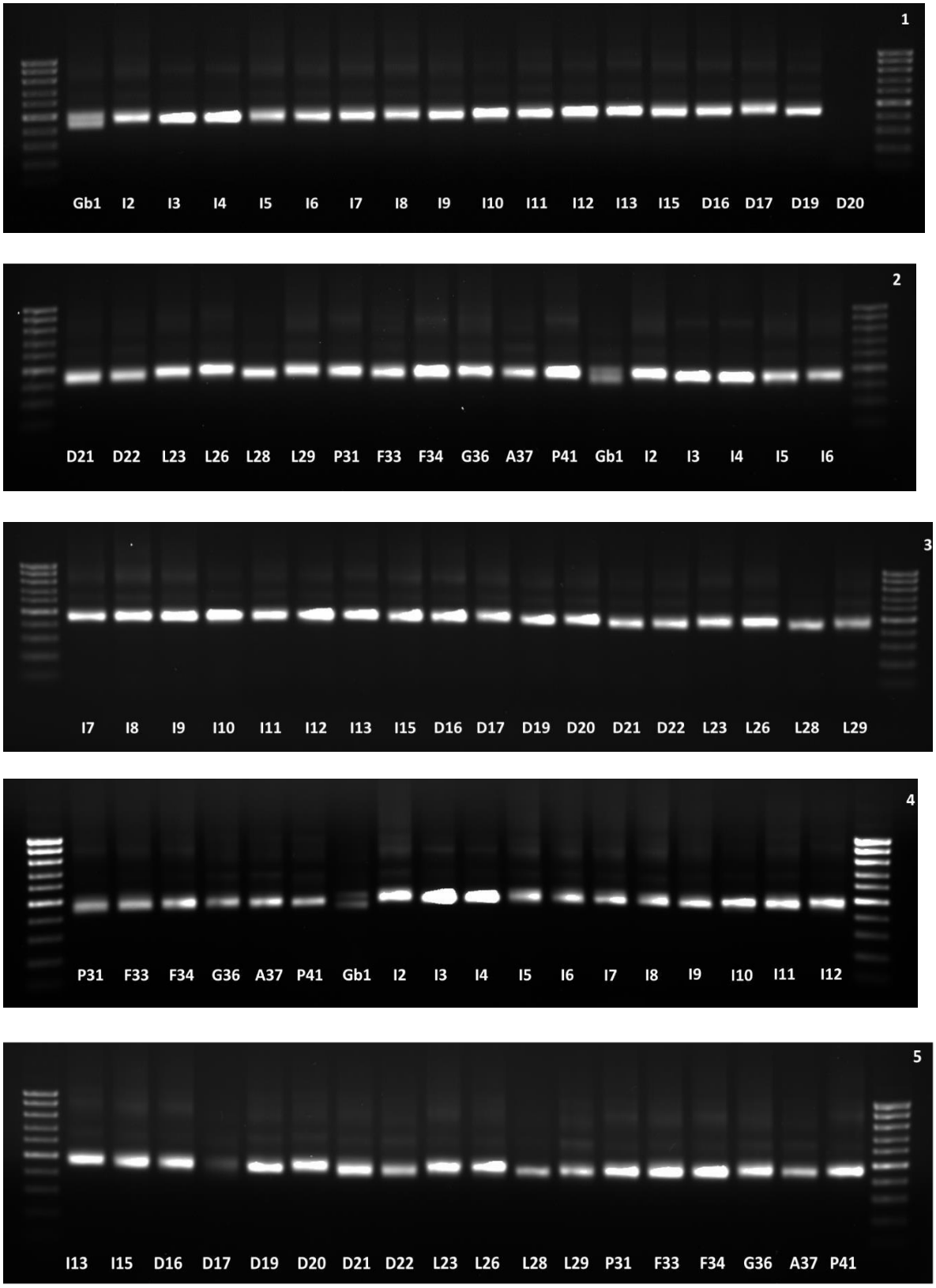


Figure 12: Agarose gel electrophoresis of PCR products using the primers ITS-S2F and ITS4R.

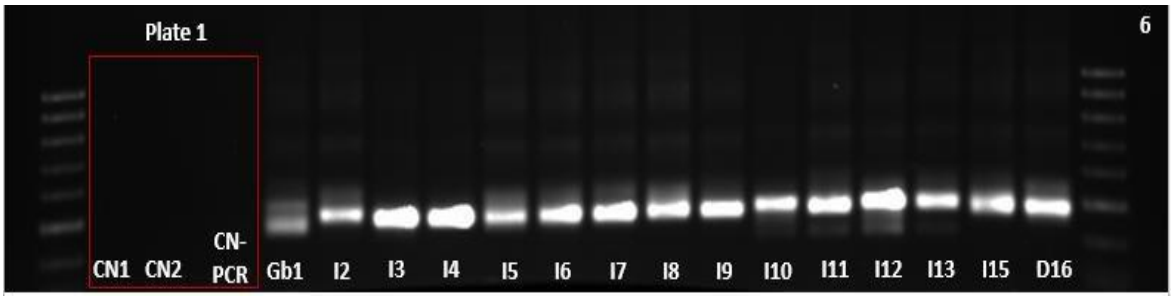
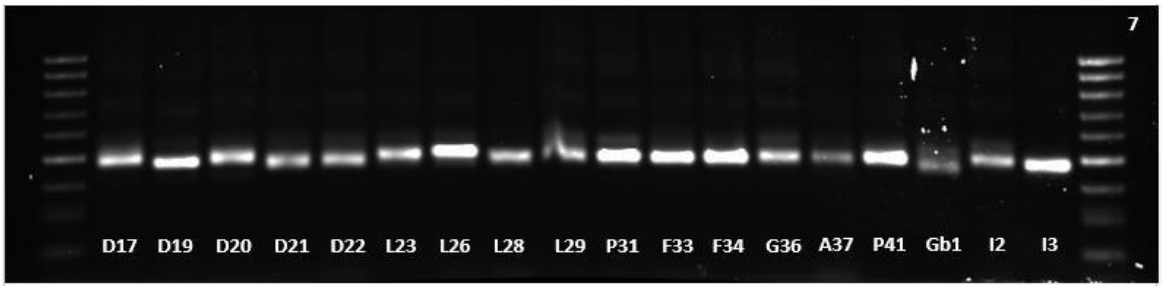
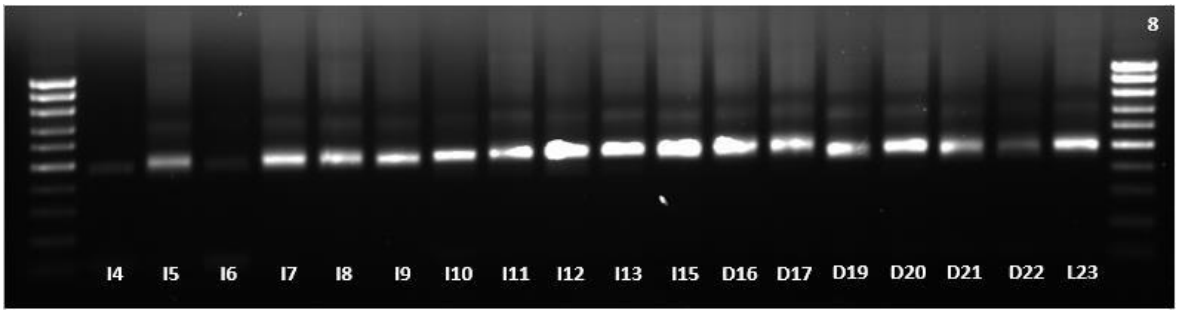
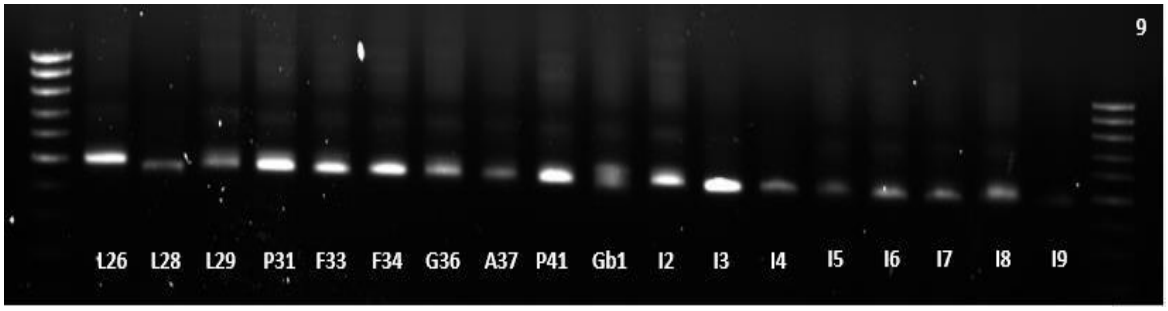
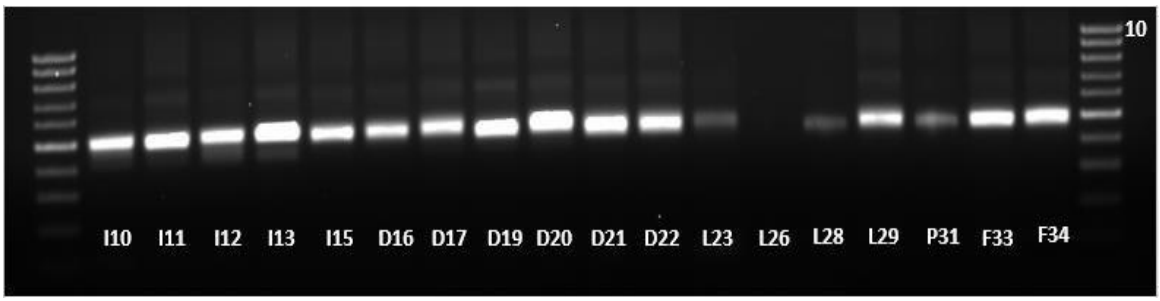


Plate 1
6
CN1
CN2
CN-PCR
Gb1
I2
I3
I4
I5
I6
I7
I8
I9
I10
I11
I12
I13
I15
D16


7
D17
D19
D20
D21
D22
L23
L26
L28
L29
P31
F33
F34
G36
A37
P41
Gb1
I2
I3


8
I4
I5
I6
I7
I8
I9
I10
I11
I12
I13
I15
D16
D17
D19
D20
D21
D22
L23


9
L26
L28
L29
P31
F33
F34
G36
A37
P41
Gb1
I2
I3
I4
I5
I6
I7
I8
I9


10
I10
I11
I12
I13
I15
D16
D17
D19
D20
D21
D22
L23
L26
L28
L29
P31
F33
F34

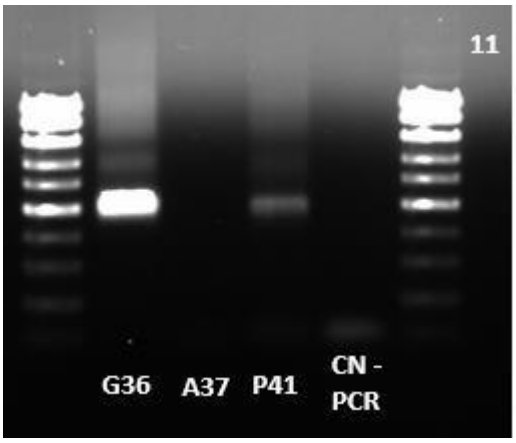


Figure 13: Agarose gel electrophoresis of PCR products using the primers ITS-u3 and ITS-u4 (Lines 2-4 correspond to negative controls of plate 1, amplified with primers ITS-S2F/ITS4R).

The PCR products were sent to CIBIO for library preparation and HTS sequencing. A total of 2,372,910 raw reads were obtained after Illumina MiSeq sequencing. Following data filtering (low quality, short reads and ambiguous base-pairs were eliminated), a number of 2,256,046 raw reads (88.8 % of the total reads) remained for subsequent analysis.

The metabarcoding approach identified taxa at the family, genus, and species level for all the 30 samples. For a complete list of the plant taxa detected, see Appendices S1 and S2 for family, S3 and S4 for genus, and S5 and S6 for species.

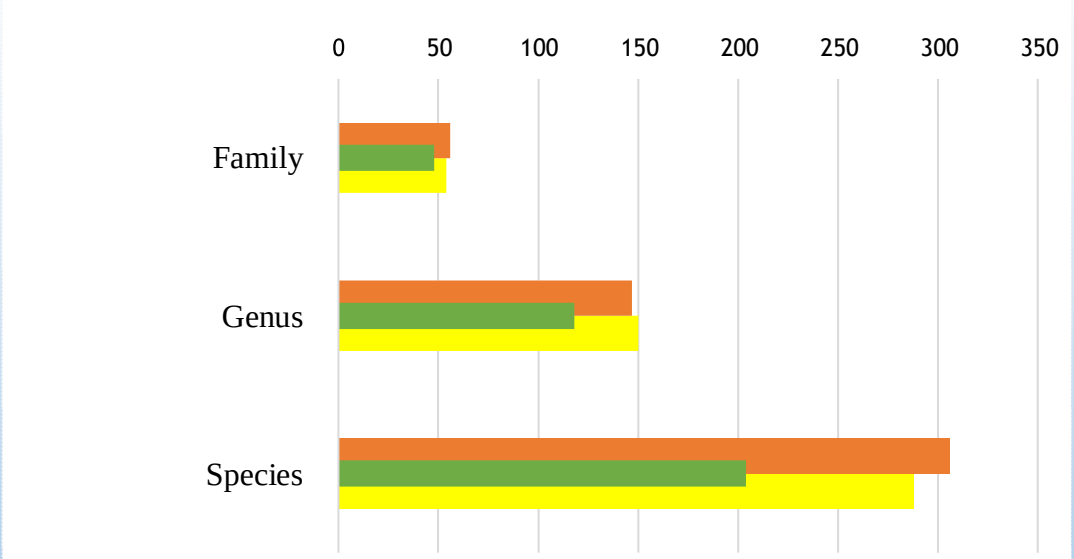
The number of taxa identified using the two primer pairs of *ITS2* were similar, with 48 families, 118 genera, and 204 species in common (Figure 14). The percentage of shared taxa between the two *ITS2* primer pairs is relatively high at the family level (87.2 %), as expected for sequences generated from the same gene, but surprisingly not so high at the genus and species levels with 79.5% and 68.7%, respectively.

The most abundant families were Fabaceae (18.4%), Brassicaceae (14.3%), Rosaceae (14%), Asteraceae (10.9%), Salicaceae (6.8%), Fagaceae (4.3%) and Ranunculaceae (2.3%), as detected by the two primer pairs ITS-S2F/ITS4R and ITS-u3/ITS-u4. Fabaceae, Rosaceae, and Ranunculaceae were also reported by Sickel et al. (2015) as the most abundant families collected by other bee species such as *O. bicornis and O. truncorum* in Germany. Most of the non-common identified families (12.8%) were rare with very low abundances ~ 0.1 %. For example, the family *Juglandaceae* was identified by the primer pair ITS-S2F and ITS4R with very low abundance (less than 0.1%) but not by ITS-u3/ITS- u4.

The most abundant genera concurrently detected by the two primer pairs were *Rubus* (7.3%), *Vicia* (7.5%), *Salix* (6.9%) and *Trifolium* (2.4%), as detected by the two primer pairs

ITS-S2F/ITS4R and ITS-u3/ITS-u4. The genera *Acer*, *Taraxacum*, and *Fraxinus* were in common with lower abundances (0.2 %, 2.2% and 3.4%, respectively). These were also reported by Richardson et al. (2015) as the most abundant families used by honey bees as a source of pollen. Most of the non-common identified families (20.5%) were rare with very low abundances (less than 0.1 %). For example, the genus *Ostrya* (in the family Betulaceae*)* was only identified by the primer pair ITS-u3/ITS-u4 and was present in just one sample(G36).

The most abundant species in common were *Brassica rapa* (5.9 %), *Hedera helix* (8 %) and *Quercus fusiformis* (5 %) as detected by the two primer pairs ITS-S2F/ITS4R and ITS-u3/ITS-u4. However, by comparing the two *ITS2* primer pairs, the latter was able to identify 18 additional species than the former. Yet,most of the samples identified by ITS-S2F/ITS4R presented higher abundances than the samples identified by ITS-u3/ITS-u4(taxa in common). For example, *Hedera helix* (Figure 15), a species of flowering plants in the family *Araliaceae*, was identified by using both primer pairs. When comparing the results for the same sample using both primer pairs, one can conclude that its abundance was generally higher when PCR amplification was carried out with ITS-S2F/ITS4R (with exception of I2 sample) than with ITS-u3/ITS-u4.



	Species	Genus	Family
ITS-u3 and ITS-u4	306	147	56
S2F and 4R	288	150	54
taxa in common	204	118	48

Figure 14: Number of families, genera and species identified using the two different primer pairs. Orange: taxa identified using the primer pair ITS-u3/ITS-u4; Yellow: taxa identified using the primer pair S2F/S4R; Green: Taxa in common.

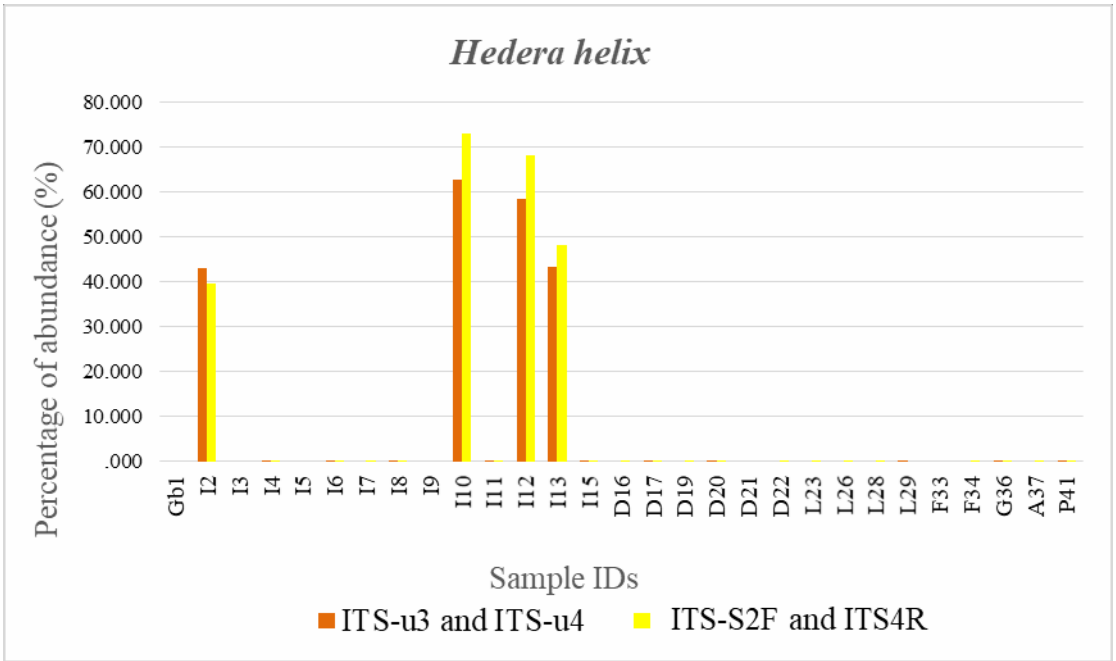


Figure 15: Percentage of abundance (%) for *Hedera helix* in the 30 analysed samples using the two different primer pairs. Orange: taxa identified using the primer pair ITS-u3 and ITS-u4 Yellow: taxa identified using the primer pair ITS-S2F and ITS4R; Green: taxa in common.

In this study, it was difficult to compare the two pairs of primers and to identify which one was more accurate regarding botanical identification. Further analyses involving microscopy-based identification of pollen, despite being a time-consuming method with limited discrimination between closely related taxa, could be helpful to verify the obtained results and conclude which primer pair allowed achieving the best identification performance (Sickel et al., 2015).

V. Conclusions

In this work, different methods were evaluated for their efficiency in extracting DNA from mixed-pollen samples collected in traps set at the entrance of hives. The method NucleoSpin revealed to be the best for DNA extraction and was therefore chosen to perform the rest of the work since it produces the best quality and yield of the extracted DNA. In addition, a variety of storage and transportation methods of pollen samples were compared for their impact on DNA quality and quantity. The results showed that the DNA extracted from the pollen samples placed in ethanol had the best quality/yield compared to the DNA extracted from the other samples with different storage conditions.

These comparisons of methods were critical for downstream DNA metabarcoding HTS with *ITS2* and therefore for botanical identification of mixed pollen samples. Two *ITS2* primer pairs, S2F/S4R and ITS-u3/ITS-u4, were employed to identify plant taxa. The number of taxa identified using these two primer pairs were similar with 48 families, 118 genera, and 204 species in common. However, the results were close enough to make the comparison difficult, although it was noted that the minor difference lies in the number of taxa that were slightly higher using ITS-u3/ITS-u4 and the abundance that was most of the time higher using ITS-S2F and ITS4R. The efficiency of the two primer pairs to identify the botanical origin of the mixed pollen could be verified by using another method such as the classical microscopic analysis, which would be interesting to perform as future work.

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Appendix

Table S1: List of families identified using the primer pair ITS-u3 and ITS-u4.

Family	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Adoxaceae	0	0	0	0	3	3	366	135	0	0	0	0	0	0	0	8	0	18	10	32	0	0	0	118	55	0	2	0	122	0
Amaranthaceae	0	1	0	0	0	0	0	0	0	5	1	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anacardiaceae	0	0	0	0	7	0	0	11	0	0	0	0	0	0	0	0	0	24	0	1	0	0	0	0	0	0	0	273	0	0
angiosperm mycorrhizal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0
Apiaceae	0	1	0	0	0	0	58	0	0	0	0	13	68	0	0	16	0	0	0	387	0	14	0	302	0	166	369	653	0	0
Aquifoliaceae	0	0	0	0	38	0	0	148	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Araliaceae	0	1740	0	1	0	1	0	0	0	3470	0	4127	1227	2	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	16
Asparagaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	70	0	0	0	0
Asphodelaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	89	0	0
Asteraceae	46	676	3	1	2	7	2	15	1	481	46	805	426	1	0	1	2932	1049	9	2432	2296	323	217	112	1	1150	2	673	2134	2
Balsaminaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0
Betulaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54	0	0	51	0	0
Boraginaceae	85	0	0	0	0	0	0	0	0	0	1046	0	0	0	0	0	0	0	0	3	2	0	0	0	0	1	9	0	201	217
Brassicaceae	1736	2	10	0	1078	42	546	147	257	952	3	1151	1	393	898	143	1	1	2290	2018	1194	1034	1201	90	889	180	757	192	10	1
Bryaceae	0	2	4	0	0	0	0	0	0	4	0	11	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cannabaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	383	0	0	0	0	0	0	0	0	0	0	0	0
Caprifoliaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	114	0	0	22	37	0	0	0	0	0	0	0	0	0	0
Caryophyllaceae	0	47	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	565	0	0	73	0	0
Cistaceae	243	0	0	0	170	11	0	250	0	0	0	0	0	1	0	110	0	0	35	0	0	0	0	0	0	0	0	0	0	1154
Cornaceae	0	0	0	0	2	3	6	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dioscoreaceae	0	2	5	0	0	0	0	0	0	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ericaceae	0	0	3	3	15	0	0	0	0	0	2	8	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Euphorbiaceae	0	324	0	0	0	0	0	0	0	2	0	0	320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	2
Fabaceae	427	0	122	84	623	72	624	716	832	1	3774	1	2	899	124	721	30	2507	2361	1782	2	61	2735	1	0	109	1578	63	10	1275

Table S1: Continued

Family	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Fagaceae	1209	0	67	74	385	285	145	361	49	0	0	1	0	192	0	0	59	1	0	0	0	0	0	0	0	83	341	193	809	702
Hyacinthaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	500	0	0
Hydrangeaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	217	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypericaceae	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	98	0	0	0	5	0	0	0	0
Iridaceae	106	0	1	0	57	0	0	8	3	0	0	0	0	7	61	10	0	0	122	20	56	74	94	0	53	9	0	0	0	0
Lamiaceae	14	101	45	10	0	1	0	19	1	305	313	754	125	3	2	99	112	99	8	26	1	2	1	0	2	13	2	2	13	2
Lythraceae	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malvaceae	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0
Microthamniales	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oleaceae	0	0	0	0	932	269	994	739	237	1	0	0	0	0	671	0	184	0	0	0	237	0	0	0	457	2	0	0	16	0
Orobanchaceae	0	1	0	0	0	0	0	0	0	0	0	0	602	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paeoniaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	352	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Papaveraceae	653	0	4	0	0	2	0	0	37	0	0	0	0	0	0	331	0	1	3	0	0	4	2	9	0	242	525	0	3	0
Pinaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	3	1	529	0	0	1	0	0	5	0	0	0
Plantaginaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	91	143	0	87	0	0	0
Platanaceae	0	0	0	0	0	1	162	1	0	0	0	0	0	1373	0	0	0	0	0	0	0	0	0	0	0	0	0	0	270	1
Poaceae	0	0	0	0	0	0	0	0	0	4	5	7	1	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Polygonaceae	0	0	0	0	0	0	105	0	1	0	0	0	0	0	0	0	0	0	0	0	0	66	0	189	0	0	0	0	0	0
Pteridaceae	0	1	0	0	0	0	0	0	0	10	0	15	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculaceae	0	661	6	17	219	57	231	1	261	5	1	12	0	93	0	5	0	1	136	11	0	1	2	346	0	6	2	1209	12	2
Resedaceae	234	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhamnaceae	0	0	0	0	0	49	0	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rosaceae	15	0	1466	416	928	965	86	697	556	2	1184	2	0	530	1292	2027	338	1280	1182	574	692	0	55	205	659	1430	1372	195	615	8
Salicaceae	0	0	1	0	317	30	22	1	479	0	1	1	0	1825	1321	1	0	0	0	0	1826	7	0	470	1147	0	2	3	2	1339

Table S1: Continued

Family	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Sapindaceae	0	0	0	0	3	0	0	31	11	0	0	0	0	0	571	0	0	0	0	0	0	0	0	0	0	0	23	0	663	0
Scrophulariaceae	10	276	1	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0	0	0	0	0	0	0	146	0	0	0	1
Solanaceae	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Styracaceae	0	0	0	0	295	268	0	526	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Syringa environmental sample	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	163	0	0	0	0	0	0	0	15	0
Ulmaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61	0	0	0	0	0
Verbenaceae	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitaceae	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S2: List of families identified using the primer pair ITS-S2F and ITS4R.

Family	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I32	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Adoxaceae	0	0	0	0	0	0	241	68	1	0	0	0	0	0	0	0	3	1	4	4	9	0	0	0	6	38	0	0	0	7	0
Anacardiaceae	0	0	0	0	1	0	0	7	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	67	0	0
angiosperm mycorrhizal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
Apiaceae	0	0	0	0	0	1	32	0	0	0	0	0	0	26	0	1	5	0	1	0	124	0	10	0	8	0	49	108	141	0	0
Aquifoliaceae	0	0	0	0	4	2	0	84	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Araliaceae	0	622	0	1	0	1	2	1	0	0	1369	0	1618	405	0	1	0	0	1	0	2	0	0	1	0	0	0	1	0	0	0
Asparagaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	61	0	0	0	0
Asphodelaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	101	0	0
Asteraceae	0	315	3	0	0	31	0	4	0	8	203	19	293	146	10	0	0	1977	323	4	877	332	181	192	4	1	245	0	210	176	0
Balsaminaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0
Betulaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41	0	0	6	0	0
Boraginaceae	10	0	0	0	0	2	0	0	2	0	0	721	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	14	3
Brassicaceae	1066	1	3	0	137	45	276	76	163	6	276	0	298	0	263	735	43	1	0	1717	744	299	812	1524	6	626	45	172	22	36	1
Cannabaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	142	0	0	0	0	0	0	0	0	0	0	0	0
Caprifoliaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	0	0	7	24	0	0	0	0	0	0	0	0	0	0
Caryophyllaceae	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	309	0	0	7	0	0
Cistaceae	139	0	0	0	18	33	0	165	0	0	0	0	0	0	0	0	22	0	0	26	0	0	0	0	0	0	0	0	0	0	377
Cornaceae	0	0	0	0	5	89	118	35	6	0	0	0	0	0	0	0	7	0	0	1	6	0	0	0	0	0	0	0	0	0	0
Ebenaceae	0	0	0	0	0	6	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ericaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0	0
Euphorbiaceae	0	86	0	0	0	0	0	0	0	0	0	0	0	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fabaceae	35	0	58	290	48	105	289	482	548	197	0	2260	0	0	512	88	211	20	972	1456	579	1	42	2731	0	1	35	470	6	0	338
Fagaceae	714	0	44	53	52	73	180	231	38	68	0	3	0	0	337	0	0	27	0	0	0	1	0	0	1	0	34	376	43	230	187
Geraniaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0

Table S2: Continued

Family	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I32	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Hyacinthaceae	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256	0	0
Hydrangeaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	126	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophyllaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0
Hypericaceae	0	0	0	2	0	0	0	0	0	0	0	35	0	0	0	0	0	0	0	0	0	0	89	0	0	0	1	0	0	0	0
Juglandaceae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lamiaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	384	0	0	0	1	0
Lauraceae	0	0	0	0	0	2	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
Lythraceae	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malvaceae	0	0	2	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oleaceae	0	0	1	1	59	219	543	450	100	0	0	0	0	0	0	412	0	82	0	0	1	38	0	0	0	522	1	1	0	3	0
Orobanchaceae	0	0	1	0	0	0	0	0	0	0	0	0	0	185	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paeoniaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Papaveraceae	983	0	8	0	0	0	9	0	100	39	0	0	0	0	0	0	322	3	0	10	0	0	36	10	5	0	149	308	0	2	0
Plantaginaceae	0	159	3	0	11	0	21	0	0	0	0	0	3	0	44	0	0	135	0	0	1	0	44	0	12	123	3	37	0	0	0
Platanaceae	0	0	0	0	0	0	103	0	0	0	0	0	0	0	777	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0
Poaceae	0	0	0	0	0	18	0	0	0	0	0	33	0	0	0	0	15	0	5	2	0	0	0	0	0	0	0	0	0	0	0
Polygonaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	83	0	8	0	1	0	0	0	0
Ranunculaceae	0	212	2	54	24	93	127	0	132	7	4	3	4	0	44	0	1	1	0	63	4	0	0	0	35	0	2	0	376	0	0
Resedaceae	54	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Rhamnaceae	0	0	0	0	2	39	0	41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rosaceae	2	0	357	399	75	567	45	490	308	976	0	600	1	0	283	1190	686	144	365	356	197	60	0	59	13	65	399	460	40	26	1
Salicaceae	0	0	0	0	21	18	13	2	239	0	0	0	0	0	1128	1078	0	0	0	1	0	230	0	0	40	710	0	0	0	0	425
Sapindaceae	0	0	0	0	0	0	0	25	11	0	0	0	0	0	0	463	0	0	0	0	0	0	0	0	0	0	0	21	0	38	0
Saxifragaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	0	0	0

Table S2: Continued

Family	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I32	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Scrophulariaceae	4	191	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	21	0	0	0	0	0	0	0	0	34	0	0	0	0
Solanaceae	0	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Styracaceae	0	0	1	0	18	209	0	405	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulmaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	0	0
Verbenaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitaceae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S3: List of genera identified using the primer pair ITS-u3 and ITS-u4.

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Acer	0	0	0	0	3	0	0	31	11	0	0	0	0	0	70	0	0	0	0	0	0	0	0	0	0	0	23	0	1	0
Aegopodium	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	237	0	0	0	0	0	0	0	0	0	0
Aesculus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	501	0	0	0	0	0	0	0	0	0	0	0	0	0	662	0
Alliaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0
Alnus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42	0	0	0	0	0
Alternanthera	0	1	0	0	0	0	0	0	0	5	1	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amorpha	0	0	0	0	0	0	0	0	0	0	264	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Angelica	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	4	0	0	0	149	0	0	0	0	0	0	0	0	0	0
angiosperm mycorrhizal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0
Anthriscus	0	0	0	0	0	0	58	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	101	0	0	18	0	0	0
Aria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asparagus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	69	0	0	0	0
Asparagus (e.s)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Asphodelus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	89	0	0
Barbarea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	145	0	0	0	0	0	0	0	0	0
Bellevalia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0
Bidens	0	0	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachymenium	0	2	4	0	0	0	0	0	0	4	0	11	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brassica	1679	2	9	0	1060	33	3	147	120	1	1	0	1	257	898	143	1	0	2289	2007	1041	1032	1201	89	887	180	0	3	1	0
Buddleja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	146	0	0	0	0
Cannabis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	383	0	0	0	0	0	0	0	0	0	0	0	0
Capsella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	6	0	0	0	0	0	0	8	0	0
Carpinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	0	0
Carum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	176	0	0	0	0	0	0

Table S3: Continued

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Castanea	0	0	67	74	43	43	35	32	37	0	0	1	0	0	0	0	59	0	0	0	0	0	0	0	0	83	0	0	13	0
Centaurea	0	28	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	9	199	0	321	216	0	0	0	0	0	0	0
Cercis	0	0	0	0	548	1	0	676	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	57	0	2
Cercis (e.s)	0	0	0	0	38	0	0	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Chaerophyllum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0
Chelidonium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	8	0	0	341	0	1	0
Chelidonium (e.s)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	184	0	2	0
Cichorium	0	173	0	0	0	0	0	0	0	1	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cirsium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	19	0	0	0	0	0	0	0	0	0	0	0	0
Cistus	243	0	0	0	15	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1154
Clematis	0	661	6	17	0	16	3	1	7	5	1	11	0	0	0	0	0	0	53	1	0	0	0	0	0	6	0	0	1	2
Colutea	141	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conopodium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	351	0	0	0
Conringia	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Cornus	0	0	0	0	2	3	6	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corylus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0
Cotoneaster	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67	0	0	563	0	0	0	0	0	0	0	0	0	0	0
Crataegus	0	0	2	1	648	113	62	149	118	0	0	0	0	207	860	16	1	0	59	1	0	0	0	0	0	0	1280	101	3	8
Crepis	0	0	0	0	0	0	0	0	0	0	0	518	0	1	0	0	0	276	0	170	0	0	0	0	0	1	0	47	0	0
Cytisus	1	0	0	0	23	18	308	0	634	0	0	0	0	0	120	0	0	0	0	0	0	0	0	0	0	0	1484	0	2	521
Datura	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dioscorea	0	2	5	0	0	0	0	0	0	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplotaxis	0	0	0	0	0	0	0	0	0	0	0	1147	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Dittrichia	0	0	0	0	0	0	0	0	0	468	0	0	426	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0

Table S3: Continued

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Echium	85	0	0	0	0	0	0	0	0	0	1046	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	217
Erica	0	0	0	0	15	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fagopyrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65	0	0	0	0	0	0	0	0
Foeniculum	0	0	0	0	0	0	0	0	0	0	0	0	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	44	0	0	0	0	0	0
Fraxinus	0	0	0	0	880	19	994	648	237	1	0	0	0	0	671	0	0	0	0	0	0	0	0	0	457	0	0	0	0	0
Galactites	0	0	3	0	1	7	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Galega	0	0	122	70	0	0	178	0	111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Genista	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	94	0	2	752
Geum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	160	0	0	0	0	0	0
Glebionis	45	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hedera	0	1740	0	1	0	1	0	0	0	3470	0	4127	1227	2	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	16
Helianthemum	0	0	0	0	155	11	0	232	0	0	0	0	0	1	0	110	0	0	35	0	0	0	0	0	0	0	0	0	0	0
Helminthotheca	0	50	0	0	0	0	0	0	0	11	18	58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heracleum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	166	0	0	0	0
Hydrangea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	217	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypericum	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	98	0	0	0	5	0	0	0	0
Hypochaeris	0	86	0	0	0	0	0	0	0	1	0	27	0	0	0	0	0	28	0	164	0	0	0	0	0	0	0	0	0	0
Ilex	0	0	0	0	38	0	0	148	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Impatiens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0
Iris	106	0	1	0	57	0	0	8	3	0	0	0	0	7	61	10	0	0	122	20	56	74	94	0	53	9	0	0	0	0
Krigia	0	55	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	27	0	112	0	0	0	0	0	0	0	0	0	1
Lathyrus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	6	0
Leucanthemum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61	0	0	0	0	0	0	0	0	0	0

Table S3: Continued

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Ligustrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	184	0	0	0	0	0	0	0	0	2	0	0	0	0
Lotus	0	0	0	0	0	0	0	0	0	0	371	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lunaria	0	0	0	0	0	0	0	0	0	0	0	0	0	53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malus	0	0	0	0	0	0	0	11	29	0	0	0	0	0	0	0	0	0	0	0	692	0	0	0	0	0	27	0	0	0
Matricaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	96	62	0	95	0	0	0	0	0	137	0	0	0	0
Melilotus	23	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mercurialis	0	324	0	0	0	0	0	0	0	2	0	0	320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	2
Myosotis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	1	8	0	201	0
Nigella	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Odontites	0	1	0	0	0	0	0	0	0	0	0	0	602	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Olea	0	0	0	0	52	250	0	91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ornithogalum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	474	0	0
Ostrya	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0
Paeonia	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	352	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paliurus	0	0	0	0	0	49	0	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Papaver	653	0	4	0	0	2	0	0	37	0	0	0	0	0	0	331	0	1	3	0	0	0	2	0	0	242	0	0	0	0
Picris	0	164	0	0	0	0	0	0	0	0	0	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pilosella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	11	0	0	0	0	0	0
Pinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	3	1	529	0	0	1	0	0	5	0	0	0
Pistacia	0	0	0	0	7	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	273	0	0
Platanus	0	0	0	0	0	1	162	1	0	0	0	0	0	1373	0	0	0	0	0	0	0	0	0	0	0	0	0	0	270	1
Pleioblastus	0	0	0	0	0	0	0	0	0	4	5	7	1	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Populus	0	0	0	0	0	0	2	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	20
Potentilla	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	119	0	0	0	0	0	0	0	0	0	27	0	0	0	0

Table S3: Continued

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Poterium	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus	0	0	0	0	113	72	1	199	72	0	0	0	0	192	0	0	0	0	0	0	0	0	0	0	659	0	0	20	609	0
Prunus (e.s)	0	0	0	0	9	8	0	57	3	0	0	1	0	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
Pteris	0	1	0	0	0	0	0	0	0	10	0	15	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Punica	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyracantha	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	288	0	0	157	0	0	0	0	0	0	0	0	0	0	0
Pyrus	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	74	0	0
Quercus	1209	0	0	0	342	242	110	329	12	0	0	0	0	192	0	0	0	1	0	0	0	0	0	0	0	0	341	193	796	702
Ranunculus	0	0	0	0	219	24	228	0	254	0	0	1	0	93	0	5	0	1	83	10	0	1	2	346	0	0	2	1209	11	0
Raphanus	3	0	1	0	17	9	543	0	137	951	2	4	0	83	0	0	0	1	0	5	0	1	0	0	2	0	757	181	0	0
Rapistrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Reichardia	0	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Reseda	234	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	0	1	0	0	0	0	0	0	0	0	0	0
Robinia	121	0	0	0	0	0	82	0	0	0	0	0	0	0	0	4	0	0	14	0	0	0	0	0	0	0	0	0	0	0
Robinia (e.s)	91	0	0	0	0	0	34	0	0	0	0	0	0	0	0	2	0	0	7	0	0	0	0	0	0	0	0	0	0	0
Rosa	15	0	0	0	102	173	2	152	0	0	0	0	0	7	0	604	0	0	94	7	0	0	0	0	0	0	0	0	0	0
Rosa (e.s)	0	0	0	0	0	2	0	2	0	0	0	0	0	2	0	322	0	0	40	4	0	0	0	0	0	0	0	0	0	0
Rubus	0	0	1464	415	21	574	21	101	334	2	1184	1	0	4	0	584	337	1279	269	562	0	0	55	1	0	1403	0	0	0	0
Rumex	0	0	0	0	0	0	105	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	189	0	0	0	0	0	0
Salix	0	0	1	0	317	30	20	1	475	0	1	1	0	1825	1321	1	0	0	0	0	1826	7	0	470	1131	0	2	3	2	1319
Sambucus	0	0	0	0	3	3	366	135	0	0	0	0	0	0	0	7	0	18	8	32	0	0	0	0	0	0	2	0	0	0
Scandix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0
Senecio	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	626	0	0

Table S3: Continued

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Silene	0	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sinapis	53	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1	0	0	1	0	0	0	0	0	0
Smyrnium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	622	0	0
Sorbus	0	0	0	0	35	0	0	26	0	0	0	0	0	0	425	27	0	0	0	0	0	0	0	0	0	0	65	0	0	0
Stachys	0	101	45	10	0	0	0	0	1	305	313	754	125	3	2	99	112	99	8	26	1	2	1	0	2	13	2	2	13	2
Stellaria	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	565	0	0	73	0	0
Styrax	0	0	0	0	295	268	0	526	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sulla	0	0	0	0	0	0	0	0	0	0	1262	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Symphoricarpos	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	114	0	0	22	37	0	0	0	0	0	0	0	0	0	0
Syringa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	237	0	0	0	0	0	0	0	16	0
Syringa (e.s)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	163	0	0	0	0	0	0	0	15	0
Taraxacum	0	0	0	0	1	0	0	9	1	0	1	7	0	0	0	0	0	0	0	1	2295	2	1	101	1	0	2	0	2133	0
Thymus	14	0	0	0	0	1	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tilia	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0
Trebouxia	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium	0	0	0	14	13	13	10	0	78	0	1876	0	0	0	0	3	0	2	0	0	0	3	0	0	0	0	0	0	0	0
Trifolium (e.s)	0	0	0	0	0	5	12	0	9	1	0	0	0	3	0	711	30	1576	0	102	0	58	0	0	0	109	0	0	0	0
Trigonella	50	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tripleurospermum	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2819	637	0	1630	0	0	0	0	0	1012	0	0	0	0
Ulex	0	0	0	0	0	0	0	0	0	0	0	0	0	896	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulmus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61	0	0	0	0	0
Vaccinium	0	0	3	3	0	0	0	0	0	0	2	8	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Verbascum	10	276	1	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0	0	0	0	0	0	0	0	0	0	0	1
Verbena	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S2: Continued

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Veronica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	91	143	0	87	0	0	0
Viburnum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	118	55	0	0	0	122	0
Vicia	0	0	0	0	1	0	0	0	0	0	0	1	1	0	3	1	0	929	2340	1680	0	0	2735	0	0	0	0	0	0	0
Vitis	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xanthium	0	71	0	0	0	0	0	0	0	0	0	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S4: List of families identified using the primer pair ITS-S2F and ITS4R.

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I32	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Acer	0	0	0	0	0	0	0	24	10	0	0	0	0	0	0	72	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0
Achillea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0
Aegopodium	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	4	0	1	0	124	0	0	0	0	0	0	0	0	0	0
Aesculus	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	391	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0
Agoseris	0	7	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	12	0	15	0	0	0	0	0	0	0	0	0	0
Ajuga	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Alnus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0
Alopecurus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amorpha	0	0	0	0	0	0	0	0	0	0	0	115	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Angiosperm mycorrhizal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
Anthriscus	0	0	0	0	0	0	32	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	11	0	0	0
Arctium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0
Aria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asparagus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	61	0	0	0	0
Asphodelus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	101	0	0
Barbarea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	138	0	0	0	0	0	0	0	0	0
Bellevalia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0
Bellis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Bidens	0	0	0	0	0	0	0	0	0	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Borago	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brassica	1022	0	3	0	135	28	2	76	65	6	0	0	0	0	172	733	42	0	0	1716	744	160	812	1524	6	626	45	0	0	0	0
Buddleja	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0
Calluna	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
Cannabis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	142	0	0	0	0	0	0	0	0	0	0	0	0

Table S4: Continued

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I32	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Capsella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0
Cardamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	36	0
Carpinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Carum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0
Castanea	0	0	44	53	6	31	43	27	20	68	0	3	0	0	0	0	0	27	0	0	0	0	0	0	0	0	34	0	0	1	0
Centaurea	0	8	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	3	86	0	166	192	0	0	0	0	0	0	0
Cercis	0	0	0	0	45	1	0	480	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Chaerophyllum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chelidonium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0	5	0	0	308	0	2	0
Cichorium	0	83	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cirsium	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	10	8	0	13	0	4	0	0	0	0	0	0	0	0
Cistus	139	0	0	0	2	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	377
Clematis	0	212	2	53	0	14	0	0	7	7	4	2	4	0	0	0	0	0	0	32	0	0	0	0	0	0	2	0	0	0	0
Conopodium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	97	0	0	0
Cornus	0	0	0	0	5	89	118	35	6	0	0	0	0	0	0	0	7	0	0	1	6	0	0	0	0	0	0	0	0	0	0
Corylus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0
Cotoneaster	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0
Crataegus	0	0	0	0	44	88	26	99	58	1	0	0	0	0	110	768	6	0	0	37	0	0	0	0	0	1	0	433	15	0	1
Crepis	0	46	0	0	0	1	0	0	0	0	0	0	175	0	10	0	0	0	96	0	61	0	0	0	0	0	0	0	21	0	0
Cydonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cytisus	0	0	0	0	1	23	139	0	432	0	0	0	0	0	0	87	0	0	0	0	0	0	0	3	0	1	0	441	0	0	196
Dactylis	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	9	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Datura	0	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diospyros	0	0	0	0	0	6	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S4: Continued

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I32	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Diplotaxis	0	1	0	0	0	3	0	0	0	0	0	0	298	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Dittrichia	0	0	0	0	0	0	0	0	0	0	200	0	0	145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Echium	10	0	0	0	0	0	0	0	0	0	0	721	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Erica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Fagopyrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	83	0	0	0	0	0	0	0	0
Fallopia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Foeniculum	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
Fraxinus	0	0	1	1	52	17	543	394	100	0	0	0	0	0	0	412	0	1	0	0	1	0	0	0	0	522	0	0	0	0	0
Galactites	0	0	3	0	0	28	0	1	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Galega	0	0	58	204	0	0	79	0	68	197	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Genista	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	0	0	142
Geranium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0
Geum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0
Halimium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hedera	0	622	0	1	0	1	2	1	0	0	1369	0	1618	405	0	1	0	0	1	0	2	0	0	1	0	0	0	1	0	0	0
Helianthemum	0	0	0	0	16	33	0	146	0	0	0	0	0	0	0	0	22	0	0	26	0	0	0	0	0	0	0	0	0	0	0
Helminthotheca	0	8	0	0	0	0	0	0	0	0	1	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heracleum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	9	0	0	0	49	0	0	0	0
Hydrangea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	126	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypericum	0	0	0	2	0	0	0	0	0	0	0	35	0	0	0	0	0	0	0	0	0	0	89	0	0	0	1	0	0	0	0
Hypochaeris	0	43	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	22	0	93	0	0	0	0	0	0	0	0	0	0
Ilex	0	0	0	0	4	2	0	84	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Impatiens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0

Table S4: Continued

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I32	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Juglans	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kickxia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laurus	0	0	0	0	0	2	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
Leontodon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leucanthemum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	20	0	0	0	0	0	0	0	0	0	0
Ligustrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	81	0	0	0	0	0	0	0	0	1	0	0	0	0
Lolium	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
Loncomelos	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lotus	0	0	0	0	0	0	0	0	0	0	0	164	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lunaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malus	0	0	0	0	0	3	0	8	27	0	0	0	0	0	0	0	0	0	0	0	0	60	0	0	0	0	0	13	0	0	0
Matricaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	90	21	0	34	0	0	0	0	0	16	0	0	0	0
Mercurialis	0	86	0	0	0	0	0	0	0	0	0	0	0	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myosotis	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	14	0
Nigella	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Odontites	0	0	1	0	0	0	0	0	0	0	0	0	0	185	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Olea	0	0	0	0	7	202	0	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ornithogalum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	225	0	0
Paeonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paliurus	0	0	0	0	0	13	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Papaver	983	0	8	0	0	0	9	0	100	39	0	0	0	0	0	0	322	3	0	10	0	0	0	10	0	0	149	0	0	0	0
Phacelia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0
Physocarpus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Picris	0	80	0	0	0	1	0	0	0	0	0	0	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S4: Continued

Genus	Gb	I2	I3	I4	I5	I6	I7	I8	I9	I32	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Saxifraga	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	0	0	0
Scandix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Senecio	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	188	0	0
Silene	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sinapis	16	0	0	0	0	0	0	0	2	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Smyrnium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	138	0	0
Solidago	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
Sorbus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	381	9	0	0	0	0	0	0	0	0	0	0	14	0	0	0
Stellaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	309	0	0	7	0	0
Styrax	0	0	1	0	18	209	0	405	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sulla	0	0	0	0	0	0	0	0	0	0	0	711	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Symphoricarpos	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	0	0	7	24	0	0	0	0	0	0	0	0	0	0
Syringa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0	1	0	3	0
Taraxacum	0	0	0	0	0	0	0	3	0	0	0	0	2	0	0	0	0	0	0	0	0	332	0	0	3	1	0	0	0	175	0
Tilia	0	0	2	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Torminalis	0	0	0	0	8	0	0	71	0	0	0	0	0	0	0	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium	0	0	0	84	2	6	8	1	40	0	0	1270	0	0	0	0	44	0	57	0	3	0	6	0	0	0	1	0	0	0	0
Trifolium (e.s)	0	0	0	0	0	2	2	0	6	0	0	0	0	0	0	0	165	20	582	0	28	0	36	0	0	0	33	0	0	0	0
Trigonella	7	0	0	0	0	73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tripleurospermum	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1868	164	0	555	0	0	0	1	0	229	0	0	0	0
Ulex	0	0	0	0	0	0	0	1	0	0	0	0	0	0	512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulmus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	0	0
uncultured Quercus pubescens from ectomycorrhiza	1	0	0	0	7	7	29	33	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	43	3
Verbascum	4	191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S4: Continued

Genus	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I32	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Verbena	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	123	0	26	0	0	0
Viburnum	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	38	0	0	0	7	0
Vicia	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	333	1448	548	1	0	2728	0	0	1	0	0	0	0
Vitis	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xanthium	0	29	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S5: List of species identified using the primer pair ITS-u3 and ITS-u4.

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Acer monspessulanum	0	0	0	0	3	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acer x pseudoheldreichii	0	0	0	0	0	0	0	20	11	0	0	0	0	0	70	0	0	0	0	0	0	0	0	0	0	0	23	0	1	0
Aegopodium podagraria	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	237	0	0	0	0	0	0	0	0	0	0
Aesculus x carnea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	501	0	0	0	0	0	0	0	0	0	0	0	0	0	662	0
Alliaria petiolata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0
Alnus hirsuta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42	0	0	0	0	0
Alternanthera sp. XF34	0	1	0	0	0	0	0	0	0	5	1	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amorpha nana	0	0	0	0	0	0	0	0	0	0	264	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Angelica sylvestris	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Angelica venenosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	149	0	0	0	0	0	0	0	0	0	0
Angiosperm mycorrhizal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0
Anthriscus velutina	0	0	0	0	0	0	58	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	101	0	0	18	0	0	0
Aria nivea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asparagus (e.s)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Asparagus officinalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0	0	0
Asparagus oligoclonos	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0
Asphodelus aestivus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	89	0	0
Barbarea vulgaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	145	0	0	0	0	0	0	0	0	0
Bellevalia romana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0
Bidens discoidea	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bidens frondosa	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bidens vulgata	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachymenium longicolle	0	2	4	0	0	0	0	0	0	4	0	11	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brassica juncea	81	0	4	0	29	0	0	0	0	0	0	0	0	0	14	5	0	0	56	6	33	23	54	0	26	2	0	0	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Brassica nigra	70	2	0	0	70	27	2	0	0	1	0	0	0	0	0	0	0	0	0	1598	0	46	5	89	0	2	0	3	0	0
Brassica oleracea	566	0	2	0	289	0	0	0	0	0	0	0	0	42	294	41	0	0	934	121	351	391	411	0	0	61	0	0	0	0
Brassica rapa	962	0	3	0	672	6	1	147	120	0	1	0	1	215	590	97	1	0	1299	282	657	572	731	0	861	115	0	0	1	0
Buddleja davidii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	55	0	0	0	0
Buddleja officinalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	91	0	0	0	0
Cannabis sativa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	383	0	0	0	0	0	0	0	0	0	0	0	0
Capsella grandiflora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	6	0	0	0	0	0	0	8	0	0
Carpinus polyneura	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	0	0
Carum carvi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	176	0	0	0	0	0	0
Castanea dentata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0
Castanea sativa	0	0	67	74	43	43	35	32	37	0	0	1	0	0	0	0	59	0	0	0	0	0	0	0	0	83	0	0	0	0
Centaurea cyanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	199	0	1	216	0	0	0	0	0	0	0
Centaurea nervosa	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	295	0	0	0	0	0	0	0	0
Centaurea nigrescens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centaurea solstitialis	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centaurea x moncktonii	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0
Cercis canadensis	0	0	0	0	40	0	0	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
Cercis (e.s)	0	0	0	0	38	0	0	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Cercis occidentalis	0	0	0	0	508	1	0	629	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	52	0	2
Cercis siliquastrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaerophyllum hirsutum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0
Chaerophyllum magellense	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0
Chelidonium (e.s)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	184	0	2	0
Chelidonium majus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	8	0	0	341	0	1	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Cichorium glandulosum	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cichorium intybus	0	168	0	0	0	0	0	0	0	1	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cichorium spinosum	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cirsium arvense	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	19	0	0	0	0	0	0	0	0	0	0	0	0
Cistus albidus	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Cistus creticus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cistus ladanifer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1146
Cistus salviifolius	140	0	0	0	15	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Clematis armandii	0	398	6	5	0	10	2	0	4	3	0	5	0	0	0	0	0	0	7	0	0	0	0	0	0	4	0	0	0	1
Clematis fasciculiflora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	43	1	0	0	0	0	0	0	0	0	1	0
Clematis gracilifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Clematis terniflora	0	263	0	12	0	6	1	1	3	2	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1
Colutea arborescens	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colutea melanocalyx	77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conopodium majus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	351	0	0	0
Conringia orientalis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Cornus sanguinea	0	0	0	0	2	3	6	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corylus avellana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0
Cotoneaster conspicuus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	0	0	345	0	0	0	0	0	0	0	0	0	0	0
Cotoneaster coriaceus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	22	0	0	0	0	0	0	0	0	0	0	0
Cotoneaster dammeri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	196	0	0	0	0	0	0	0	0	0	0	0
Crataegus babakhanloui	0	0	2	1	612	107	60	144	110	0	0	0	0	200	829	0	1	0	0	1	0	0	0	0	0	0	1225	98	3	7
Crataegus erythropoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	59	0	0	0	0	0	0	0	0	0	0	0
Crataegus heldreichii	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Crataegus monogyna	0	0	0	0	4	0	2	2	2	0	0	0	0	3	5	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1
Crataegus monogyna x Crataegus suksdorfii	0	0	0	0	29	6	0	3	6	0	0	0	0	3	26	0	0	0	0	0	0	0	0	0	0	0	48	3	0	0
Crataegus nigra	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Crepis capillaris	0	0	0	0	0	0	0	0	0	0	0	311	0	0	0	0	0	182	0	108	0	0	0	0	0	1	0	0	0	0
Crepis nicaeensis	0	0	0	0	0	0	0	0	0	0	0	207	0	0	0	0	0	94	0	62	0	0	0	0	0	0	0	0	0	0
Crepis sancta	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	47	0	0
Cytisus arboreus	0	0	0	0	11	12	192	0	347	0	0	0	0	0	72	0	0	0	0	0	0	0	0	0	0	0	929	0	2	296
Cytisus maurus	0	0	0	0	0	0	3	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	2
Cytisus multiflorus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Cytisus scoparius	1	0	0	0	12	6	113	0	277	0	0	0	0	0	48	0	0	0	0	0	0	0	0	0	0	0	552	0	0	219
Datura stramonium	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dioscorea deltoidea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dioscorea pentaphylla	0	2	5	0	0	0	0	0	0	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplotaxis tenuifolia	0	0	0	0	0	0	0	0	0	0	0	1147	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Dittrichia viscosa	0	0	0	0	0	0	0	0	0	468	0	0	426	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Echium plantagineum	85	0	0	0	0	0	0	0	0	0	1046	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	217
Erica arborea	0	0	0	0	15	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fagopyrum esculentum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0	0	0	0
Fagopyrum homotropicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0	0	0	0	0	0	0
Foeniculum vulgare	0	0	0	0	0	0	0	0	0	0	0	0	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragaria chiloensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0	0	0
Fragaria virginiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	25	0	0	0	0	0	0
Fraxinus angustifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0
Fraxinus ornus	0	0	0	0	880	19	994	648	237	1	0	0	0	0	671	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Fraxinus potamophila	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	441	0	0	0	0	0
Galactites tomentosus	0	0	3	0	1	7	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Galega officinalis	0	0	122	70	0	0	178	0	111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Genista anglica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	63	0	2	0
Genista florida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Genista hystrix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	269
Genista pilosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0	0
Genista tenera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	481
Geum rivale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	160	0	0	0	0	0	0
Glebionis segetum	45	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hedera helix	0	1740	0	1	0	1	0	0	0	3470	0	4127	1227	2	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	16
Helianthemum asperum	0	0	0	0	54	4	0	66	0	0	0	0	0	1	0	43	0	0	15	0	0	0	0	0	0	0	0	0	0	0
Helianthemum nummularium	0	0	0	0	20	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helianthemum raskebdanae	0	0	0	0	81	7	0	133	0	0	0	0	0	0	0	61	0	0	20	0	0	0	0	0	0	0	0	0	0	0
Helianthemum sauvagei	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helianthemum violaceum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helminthotheca echioides	0	50	0	0	0	0	0	0	0	11	18	58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heracleum dissectum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	166	0	0	0	0
Heracleum trachyloma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0
Hydrangea macrophylla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	123	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrangea serrata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	94	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypericum maculatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0
Hypericum perforatum	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	80	0	0	0	5	0	0	0	0
Hypochaeris arachnoides	0	84	0	0	0	0	0	0	0	1	0	27	0	0	0	0	0	27	0	162	0	0	0	0	0	0	0	0	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Hypochaeris glabra	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0
Ilex aquifolium	0	0	0	0	38	0	0	148	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Impatiens capensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0
Iris loczyi	106	0	1	0	57	0	0	8	3	0	0	0	0	7	61	10	0	0	122	20	56	74	94	0	53	9	0	0	0	0
Krigia sp. VanNeste 472	0	55	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	27	0	112	0	0	0	0	0	0	0	0	0	1
Lathyrus latifolius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	6	0
Leucanthemum laciniatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61	0	0	0	0	0	0	0	0	0	0
Ligustrum ovalifolium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	184	0	0	0	0	0	0	0	0	2	0	0	0	0
Lotus subbiflorus	0	0	0	0	0	0	0	0	0	0	371	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lunaria annua	0	0	0	0	0	0	0	0	0	0	0	0	0	53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malus domestica	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0
Malus micromalus	0	0	0	0	0	0	0	7	18	0	0	0	0	0	0	0	0	0	0	0	433	0	0	0	0	0	17	0	0	0
Malus orientalis	0	0	0	0	0	0	0	3	8	0	0	0	0	0	0	0	0	0	0	0	209	0	0	0	0	0	10	0	0	0
Matricaria aurea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	48	44	0	62	0	0	0	0	0	133	0	0	0	0
Matricaria chamomilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	48	18	0	33	0	0	0	0	0	4	0	0	0	0
Melilotus elegans	23	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mercurialis annua	0	324	0	0	0	0	0	0	0	2	0	0	320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	2
Myosotis stenophylla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	1	8	0	201	0
Nigella damascena	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Odontites corsicus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Odontites luteus	0	1	0	0	0	0	0	0	0	0	0	0	602	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Olea europaea	0	0	0	0	52	250	0	91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ornithogalum cuspidatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0
Ornithogalum lanceolatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	454	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Ostrya carpinifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0
Paeonia hybrid cultivar	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	193	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paeonia officinalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	159	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paliurus spina-christi	0	0	0	0	0	49	0	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Papaver bracteatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Papaver commutatum	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Papaver macrostomum	17	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0
Papaver orientale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	277	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Papaver rhoeas	633	0	4	0	0	2	0	0	36	0	0	0	0	0	0	1	0	1	2	0	0	0	2	0	0	22	0	0	0	0
Papaver sp. Yuan 2000611	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	51	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Picris hispidissima	0	164	0	0	0	0	0	0	0	0	0	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pilosella hoppeana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	11	0	0	0	0	0	0
Pinus thunbergii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	3	1	52	0	0	1	0	0	5	0	0	0
Pistacia integerrima	0	0	0	0	4	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0
Pistacia terebinthus	0	0	0	0	3	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0
Platanus cf. orientalis A11	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Platanus occidentalis	0	0	0	0	0	0	53	1	0	0	0	0	0	59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	92	1
Platanus orientalis	0	0	0	0	0	1	102	0	0	0	0	0	0	73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	168	0
Platanus rzedowskii	0	0	0	0	0	0	3	0	0	0	0	0	0	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0
Platanus x hispanica	0	0	0	0	0	0	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Pleioblastus fortunei	0	0	0	0	0	0	0	0	0	4	5	7	1	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Populus koreana	0	0	0	0	0	0	2	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	20
Potentilla anserina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	119	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potentilla reptans	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	0	0	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Poterium sanguisorba	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus avium	0	0	0	0	13	8	0	22	3	0	0	0	0	107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus cerasus	0	0	0	0	3	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Prunus davidiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0
Prunus dulcis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus elaeagrifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0
Prunus (e.s)	0	0	0	0	9	8	0	57	3	0	0	1	0	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
Prunus fordiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus laurocerasus	0	0	0	0	0	54	1	0	69	0	0	0	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	603	0
Prunus lycioides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	616	0	0	0	0	0
Prunus mahaleb	0	0	0	0	55	3	0	93	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	13	4	0
Prunus nigra	0	0	0	0	29	0	0	63	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	7	1	0
Prunus pseudocerasus	0	0	0	0	13	7	0	19	0	0	0	0	0	46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus sp. EB-2002	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pteris tremula	0	1	0	0	0	0	0	0	0	10	0	15	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Punica granatum	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyracantha fortuneana	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	288	0	0	157	0	0	0	0	0	0	0	0	0	0	0
Pyrus cordata	0	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	71	0	0
Pyrus elaeagrifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Pyrus salicifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Quercus alnifolia	17	0	0	0	26	28	2	41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	64
Quercus aucheri	1190	0	0	0	104	175	20	198	10	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	11	176	0	432
Quercus buckleyi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0
Quercus cerris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Quercus cf. castaneifolia Denk 996078	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus coccifera	0	0	0	0	3	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus fusiformis	0	0	0	0	74	27	37	42	1	0	0	0	0	91	0	0	0	0	0	0	0	0	0	0	0	0	73	0	332	17
Quercus infectoria	1	0	0	0	15	0	8	7	0	0	0	0	0	44	0	0	0	0	0	0	0	0	0	0	0	0	71	0	27	63
Quercus macranthera	0	0	0	0	26	5	7	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	387	21
Quercus protoroburoides	1	0	0	0	6	0	2	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2
Quercus pubescens	0	0	0	0	76	7	24	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24
Quercus pyrenaica	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45
Quercus robur	0	0	0	0	12	0	10	10	1	0	0	0	0	47	0	0	0	0	0	0	0	0	0	0	0	0	180	0	13	34
Ranunculus bulbosus	0	0	0	0	37	24	40	0	24	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0
Ranunculus chius	0	0	0	0	0	0	3	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus garganicus	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus granatensis	0	0	0	0	0	0	64	0	218	0	0	1	0	12	0	2	0	0	5	0	0	0	2	3	0	0	0	0	0	0
Ranunculus grandifolius	0	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus lanuginosus	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus macounii	0	0	0	0	68	0	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	64	0	0
Ranunculus marginatus	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1018	0	0
Ranunculus repens	0	0	0	0	0	0	1	0	1	0	0	0	0	48	0	1	0	0	51	3	0	0	0	197	0	0	0	0	9	0
Ranunculus sardous	0	0	0	0	3	0	3	0	2	0	0	0	0	31	0	2	0	1	27	7	0	1	0	145	0	0	0	1	2	0
Ranunculus trilobus	0	0	0	0	111	0	60	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	124	0	0
Raphanus sativus	3	0	1	0	17	9	543	0	137	951	2	4	0	83	0	0	0	1	0	5	0	1	0	0	2	0	757	181	0	0
Rapistrum rugosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Reichardia picroides	0	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Reseda lutea	234	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Rhus typhina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	0	1	0	0	0	0	0	0	0	0	0	0
Robinia environmental sample spc	91	0	0	0	0	0	34	0	0	0	0	0	0	0	0	2	0	0	7	0	0	0	0	0	0	0	0	0	0	0
Robinia neomexicana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Robinia pseudoacacia	121	0	0	0	0	0	82	0	0	0	0	0	0	0	0	4	0	0	12	0	0	0	0	0	0	0	0	0	0	0
Rosa arkansana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Rosa chinensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Rosa environmental sample spc	0	0	0	0	0	2	0	2	0	0	0	0	0	2	0	322	0	0	40	4	0	0	0	0	0	0	0	0	0	0
Rosa henryi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rosa kwangtungensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rosa obtusifolia	5	0	0	0	34	34	1	49	0	0	0	0	0	0	0	26	0	0	5	0	0	0	0	0	0	0	0	0	0	0
Rosa phoenicia	0	0	0	0	32	59	0	33	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0
Rosa platyacantha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	6	2	0	0	0	0	0	0	0	0	0	0
Rosa primula	10	0	0	0	36	79	1	70	0	0	0	0	0	7	0	553	0	0	69	5	0	0	0	0	0	0	0	0	0	0
Rosa sempervirens	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rubus aff. wahlbergii MS-2014	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	15	0	29	0	0	0	0	0	0	0	0	0	0	0
Rubus bollei	0	0	607	188	9	215	8	51	140	2	478	1	0	0	0	45	133	492	102	162	0	0	0	0	0	696	0	0	0	0
Rubus hypomalacus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0
Rubus palmensis	0	0	836	227	12	358	13	50	187	0	706	0	0	0	0	46	176	781	133	243	0	0	1	0	0	702	0	0	0	0
Rubus scissoides	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	490	0	0	5	152	0	0	54	1	0	0	0	0	0	0
Rubus subcoreanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rubus ulmifolius x Rubus caesius	0	0	21	0	0	0	0	0	7	0	0	0	0	0	0	3	12	6	0	3	0	0	0	0	0	5	0	0	0	0
Rubus vigorosus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rumex acetosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	180	0	0	0	0	0	0
Rumex graminifolius	0	0	0	0	0	0	105	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Rumex thyrsiflorus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
Salix atrocinerea	0	0	0	0	100	6	5	1	189	0	0	1	0	663	528	0	0	0	0	0	703	2	0	233	449	0	0	2	0	518
Salix rehderiana	0	0	1	0	181	23	11	0	228	0	1	0	0	1116	790	1	0	0	0	0	1117	5	0	237	670	0	2	1	2	769
Salix serissima	0	0	0	0	13	0	4	0	28	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Salix songarica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0
Salix warburgii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	6	0	0	0	3	0	0	0	0	8
Salix wilsonii	0	0	0	0	23	1	0	0	30	0	0	0	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
Sambucus nigra	0	0	0	0	3	3	366	135	0	0	0	0	0	0	0	7	0	18	8	32	0	0	0	0	0	0	2	0	0	0
Scandix pecten-veneris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0
Senecio kerdousianus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	615	0	0
Senecio rupestris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0
Silene latifolia	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Silene sp. MLK-2011	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sinapis arvensis	53	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1	0	0	1	0	0	0	0	0	0
Smyrnium olusatrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	622	0	0
Sorbus aucuparia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	132	4	0	0	0	0	0	0	0	0	0	0	19	0	0	0
Sorbus bristoliensis	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sorbus chamaemespilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sorbus dumosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	2	0	0	0	0	0	0	0	0	0	0	11	0	0	0
Sorbus leyana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	88	1	0	0	0	0	0	0	0	0	0	0	12	0	0	0
Sorbus pseudofennica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	140	20	0	0	0	0	0	0	0	0	0	0	23	0	0	0
Sorbus torminalis	0	0	0	0	33	0	0	25	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stachys benthamiana	0	101	45	10	0	0	0	0	1	305	313	754	125	3	2	99	112	99	8	26	1	2	1	0	2	13	2	2	13	2
Stellaria media	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	564	0	0	71	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Stellaria neglecta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0
Styrax officinalis	0	0	0	0	295	268	0	526	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sulla coronaria	0	0	0	0	0	0	0	0	0	0	1262	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Symphoricarpos albus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Symphoricarpos occidentalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	2	3	0	0	0	0	0	0	0	0	0	0
Symphoricarpos orbiculatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99	0	0	20	34	0	0	0	0	0	0	0	0	0	0
Syringa (e.s)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	163	0	0	0	0	0	0	0	15	0
Syringa vulgaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	237	0	0	0	0	0	0	0	16	0
Taraxacum (sect. Naevosa) sp. 265-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Taraxacum sp. Lepschi 4865	0	0	0	0	1	0	0	9	1	0	1	7	0	0	0	0	0	0	0	1	2294	2	1	101	1	0	2	0	2133	0
Thymus eigii	14	0	0	0	0	1	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tilia x euchlora	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0
Trebouxia sp. PM1	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium berytheum	0	0	0	4	0	0	0	0	0	0	685	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium canescens	0	0	0	10	0	3	0	0	0	0	1191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium environmental sample spc	0	0	0	0	0	5	12	0	9	1	0	0	0	3	0	711	30	1576	0	102	0	58	0	0	0	109	0	0	0	0
Trifolium incarnatum	0	0	0	0	13	0	0	0	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium longipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Trifolium nigrescens	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium squamosum	0	0	0	0	0	10	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium suffocatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2	0	0	0	0	0	0	0	0
Trigonella anguina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trigonella balansae	50	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tripleurospermum maritimum	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2819	637	0	1630	0	0	0	0	0	1012	0	0	0	0

Table S5: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Ulex borgiae	0	0	0	0	0	0	0	0	0	0	0	0	0	171	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulex europaeus	0	0	0	0	0	0	0	0	0	0	0	0	0	157	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulex gallii	0	0	0	0	0	0	0	0	0	0	0	0	0	273	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulex parviflorus	0	0	0	0	0	0	0	0	0	0	0	0	0	295	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulmus rubra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61	0	0	0	0	0
Vaccinium ashei	0	0	3	3	0	0	0	0	0	0	2	8	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Verbascum lychnitis	10	45	1	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0	0	0	0	0	0	0	0	0	0	0	0
Verbascum phoeniceum	0	231	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Verbena officinalis	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronica chamaedrys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	91	0	0	31	0	0	0
Veronica persica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	143	0	56	0	0	0
Viburnum opulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	118	0	0	0	0	0	0
Viburnum tinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	55	0	0	0	0	0
Viburnum veitchii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	122	0
Vicia faba	0	0	0	0	1	0	0	0	0	0	0	1	1	0	3	1	0	929	2340	1679	0	0	2735	0	0	0	0	0	0	0
Vicia paucijuga	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Vitis riparia	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xanthium orientale	0	71	0	0	0	0	0	0	0	0	0	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S6: List of genera identified using the primer pair ITS-S2F and ITS4R.

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Acer monspessulanum	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acer pseudoplatanus	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acer x pseudoheldreichii	0	0	0	0	0	0	0	17	10	0	0	0	0	0	72	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0
Achillea santolina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	21	0	0
Aegopodium podagraria	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	4	0	1	0	124	0	0	0	0	0	0	0	0	0	0
Aesculus x carnea	0	0	0	0	0	0	0	1	1	0	0	0	0	0	391	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Agoseris x elata	0	7	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	12	0	15	0	0	0	0	0	0	0	0	0	0
Ajuga reptans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alnus hirsuta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	3
Alnus rubra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Alopecurus pratensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Amorpha apiculata	0	0	0	0	0	0	0	0	0	0	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amorpha nana	0	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anthriscus velutina	0	0	0	0	0	0	32	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	11	0	0	0
Arctium minus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0
Aria nivea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0
Asparagus oligoclonos	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	61	0	0	0	0
Asphodelus aestivus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Barbarea intermedia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	138	0	0	0	0	0	0	0	0	0
Barbarea verna	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bellevalia dubia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bellevalia romana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bellis annua	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bellis pusilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Bidens discoidea	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Bidens frondosa	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bidens vulgata	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Borago officinalis	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brassica juncea	15	0	0	0	5	0	0	4	0	0	0	0	0	0	25	2	0	0	54	3	5	1	136	0	12	2	0	0	0	0
Brassica maurorum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brassica nigra	30	0	0	0	8	24	2	0	0	0	0	0	0	0	0	0	0	0	1	596	0	21	2	6	0	0	0	0	154	0
Brassica oleracea	363	0	1	0	39	0	0	0	0	0	0	0	0	25	205	14	0	0	637	38	55	312	480	0	1	14	0	0	0	0
Brassica rapa	609	0	2	0	82	4	0	71	64	0	0	0	0	145	500	26	0	0	1015	107	99	468	886	0	608	29	0	0	0	0
Brassica sp. ZOOMUST	5	0	0	0	1	0	0	1	1	0	0	0	0	2	3	0	0	0	9	0	1	10	20	0	5	0	0	0	0	0
Buddleja officinalis	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0
Calluna vulgaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
Cannabis sativa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	142	0	0	0	0	0	0	0	0	0	0	0	410
Capsella grandiflora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Cardamine x insueta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carpinus orientalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carpinus polyneura	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0
Carum carvi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0
Castanea dentata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Castanea sativa	0	0	44	53	6	31	43	27	20	0	3	0	0	0	0	0	27	0	0	0	0	0	0	0	0	34	0	0	0	0
Centaurea cyanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	86	0	0	192	0	0	0	0	0	0	0
Centaurea nervosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99	0	0	0	0	0	0	0	0
Centaurea solstitialis	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centaurea x moncktonii	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	67	0	0	0	0	0	0	0	0

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Cercis siliquastrum	0	0	0	0	45	1	0	480	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaerophyllum magellense	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chelidonium majus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0	5	0	0	308	0	0	0
Cichorium intybus	0	6	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	126	144
Cichorium spinosum	0	77	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0
Cirsium arvense	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	8	0	0	0	0	0	0	0	0	0	0	0	0
Cirsium palustre	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	4	0	0	0	0	0	0	0	0
Cistus albidus	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cistus ladanifer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Cistus salviifolius	87	0	0	0	2	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0
Clematis apiifolia	0	210	2	53	0	14	0	0	7	3	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	15	0	1
Clematis delavayi	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Clematis fasciculiflora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0	0	0	0	0	0	0	0	0	0
Clematis gracilifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Clematis vitalba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conopodium majus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	97	16	0	0
Cornus sanguinea	0	0	0	0	5	89	118	35	6	0	0	0	0	0	0	7	0	0	1	6	0	0	0	0	0	0	0	0	0	0
Corylus heterophylla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0
Cotoneaster coriaceus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	1
Cotoneaster dammeri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	0
Cotoneaster poluninii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crataegus babakhanloui	0	0	0	0	39	77	24	89	57	0	0	0	0	99	725	0	0	0	0	0	0	0	0	0	1	0	385	0	0	8
Crataegus crus-galli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	25	0	0	0	0	0	0	0	0	0	0	0
Crataegus heldreichii	0	0	0	0	1	3	1	1	0	0	0	0	0	3	5	1	0	0	0	0	0	0	0	0	0	0	4	21	0	0

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Crataegus monogyna	0	0	0	0	4	8	1	9	1	0	0	0	0	8	38	0	0	0	0	0	0	0	0	0	0	0	44	0	0	0
Crataegus triflora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	12	0	0	0	0	0	0	0	0	0	0	0
Crepis bursifolia	0	46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
Crepis capillaris	0	0	0	0	0	1	0	0	0	0	0	175	0	0	0	0	0	96	0	61	0	0	0	0	0	0	0	0	0	0
Crepis sancta	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cydonia oblonga	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cytisus maurus	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	165
Cytisus multiflorus	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Cytisus scoparius	0	0	0	0	1	23	137	0	431	0	0	0	0	0	87	0	0	0	0	0	0	0	2	0	1	0	441	0	0	0
Dactylis glomerata	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	9	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Datura stramonium	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diospyros tsangii	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diospyros virginiana	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplotaxis erucoides	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplotaxis tenuifolia	0	0	0	0	0	0	0	0	0	0	0	298	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dittrichia viscosa	0	0	0	0	0	0	0	0	0	200	0	0	145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Echium plantagineum	10	0	0	0	0	0	0	0	0	0	721	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erica australis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Fagopyrum esculentum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0
Fagopyrum homotropicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	59	0	0	0	0	0	0	0	0
Fallopia convolvulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Foeniculum vulgare	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragaria chiloensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragaria virginiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Fraxinus angustifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	18	0	0	0	0	0
Fraxinus ornus	0	0	1	1	52	17	543	394	100	0	0	0	0	0	412	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Fraxinus potamophila	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	504	0	0	0	0	0
Galactites tomentosus	0	0	3	0	0	28	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Galega officinalis	0	0	58	204	0	0	79	0	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Genista anglica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	0	0	0
Genista florida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Genista hystrix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Genista tenera	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Geranium molle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Geum canadense	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
Geum virginianum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Halimium umbellatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hedera helix	0	622	0	1	0	1	2	1	0	1369	0	1618	405	0	1	0	0	1	0	2	0	0	1	0	0	0	1	0	0	0
Helianthemum apenninum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Helianthemum nummularium	0	0	0	0	16	33	0	143	0	0	0	0	0	0	0	21	0	0	24	0	0	0	0	0	0	0	0	0	0	0
Helianthemum sauvagei	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helianthemum scopulicola	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helminthotheca echioides	0	8	0	0	0	0	0	0	0	1	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heracleum dissectum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	49	0	0	0	0
Heracleum trachyloma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0
Hydrangea macrophylla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrangea serrata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	118	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypericum maculatum	0	0	0	2	0	0	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0	88	0	0	0	1	0	0	0	0

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Hypericum perforatum	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Hypericum undulatum	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypochaeris radicata	0	43	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	22	0	93	0	0	0	0	0	0	0	0	0	0
Ilex aquifolium	0	0	0	0	4	2	0	84	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Impatiens capensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0
Juglans sigillata x Juglans regia	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kickxia spuria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laurus nobilis	0	0	0	0	0	2	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leontodon filii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leucanthemum gallaecicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	20	0	0	0	0	0	0	0	0	0	0
Ligustrum ovalifolium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	81	0	0	0	0	0	0	0	0	1	0	0	0	0
Ligustrum vulgare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lolium multiflorum	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	376
Loncomelos narbonense	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lotus subbiflorus	0	0	0	0	0	0	0	0	0	0	164	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lunaria annua	0	0	0	0	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malus domestica	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0
Malus micromalus	0	0	0	0	0	3	0	3	18	0	0	0	0	0	0	0	0	0	0	0	40	0	0	0	0	0	9	0	0	0
Malus orientalis	0	0	0	0	0	0	0	5	6	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	4	0	14	0
Matricaria aurea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	14	0	12	0	0	0	0	0	13	0	0	0	0
Matricaria chamomilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	71	7	0	22	0	0	0	0	0	3	0	0	0	0
Mercurialis annua	0	86	0	0	0	0	0	0	0	0	0	0	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myosotis stenophylla	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Nigella damascena	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	1	2

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Odontites corsicus	0	0	1	0	0	0	0	0	0	0	0	0	176	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Odontites luteus	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Olea europaea	0	0	0	0	7	202	0	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ornithogalum baeticum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ornithogalum cuspidatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paeonia hybrid cultivar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paeonia lactiflora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0	376	0	0
Paliurus spina-christi	0	0	0	0	0	13	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Papaver bracteatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	49	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Papaver orientale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	272	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Papaver rhoeas	983	0	8	0	0	0	9	0	100	0	0	0	0	0	0	1	3	0	4	0	0	0	10	0	0	149	0	0	0	0
Phacelia tanacetifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	1	0	0
Physocarpus opulifolius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Picris hieracioides	0	1	0	0	0	0	0	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Picris hispidissima	0	10	0	0	0	1	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
Picris morrisonensis	0	69	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pistacia integerrima	0	0	0	0	1	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pistacia terebinthus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	25
Plantago leiopetala	0	159	3	0	11	0	21	0	0	0	0	3	0	44	0	0	135	0	0	1	0	8	0	0	0	0	11	6	0	0
Plantago media	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0
Plantago virginica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0	0	0	3	0	0	0	0
Platanus occidentalis	0	0	0	0	0	0	3	0	0	0	0	0	0	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Platanus orientalis	0	0	0	0	0	0	60	0	0	0	0	0	0	373	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Platanus rzedowskii	0	0	0	0	0	0	40	0	0	0	0	0	0	359	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Platanus x hispanica	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Populus koreana	0	0	0	0	0	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0
Potentilla anserina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potentilla reptans	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0
Poterium sanguisorba	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus arabica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
Prunus cerasus	0	0	0	0	3	4	0	20	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus davidiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Prunus dulcis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	43	0	0	0	0	0
Prunus elaeagrifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0
Prunus laurocerasus	0	0	0	0	0	19	0	0	27	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus lycioides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Prunus mahaleb	0	0	0	0	6	0	0	79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus pseudocerasus	0	0	0	0	1	7	0	46	3	0	0	0	0	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus sp. EB-2002	0	0	0	0	2	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Punica granatum	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyracantha fortuneana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	84	0	0	74	0	0	0	0	0	0	0	0	0	0	0
Pyrus amygdaliformis	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyrus salicifolia	0	0	0	0	0	0	0	1	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus aucheri	712	0	0	0	7	5	3	27	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0
Quercus buckleyi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus cerris	0	0	0	0	1	3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus coccifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
Quercus faginea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Quercus frainetto	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus fusiformis	1	0	0	0	22	22	70	109	4	0	0	0	0	284	0	0	0	0	0	0	0	0	0	1	0	0	293	0	0	0
Quercus infectoria	0	0	0	0	2	3	7	13	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0
Quercus macranthera	0	0	0	0	1	0	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Quercus pubescens	0	0	0	0	6	2	15	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Quercus pyrenaica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus robur	0	0	0	0	0	0	11	7	1	0	0	0	0	36	0	0	0	0	0	0	1	0	0	0	0	0	40	0	0	0
Ranunculus bulbosus	0	0	0	0	12	65	35	0	27	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus garganicus	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus granatensis	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus grandifolius	0	0	0	1	0	0	25	0	94	0	0	0	0	7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus marginatus	0	0	0	0	12	6	54	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus repens	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0	1	0	0	31	4	0	0	0	35	0	0	0	0	0	0
Raphanus sativus	28	0	0	0	2	14	274	0	96	276	0	0	0	50	0	0	1	0	0	0	0	0	0	0	0	0	172	0	0	0
Reichardia picroides	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	3
Reseda lutea	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Rhamnus sp. KUN 0602432	0	0	0	0	2	26	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	138	0	0
Rhus typhina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	150	0	0
Robinia neomexicana	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	222	0	0
Robinia pseudoacacia	25	0	0	0	0	0	60	0	0	0	0	0	0	0	0	2	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Rosa chinensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	6	0	0
Rosa henryi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	20	0	0
Rosa kwangtungensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	101	0	0
Rosa multiflora	0	0	0	0	1	3	0	6	0	0	0	0	0	0	0	302	0	0	29	1	0	0	0	0	0	0	0	12	0	0

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Rosa phoenicia	0	0	0	0	1	45	0	16	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	30	0	0
Rosa platyacantha	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	2	0	0
Rosa primula	2	0	0	0	7	44	2	64	4	0	0	0	0	0	0	37	0	0	23	1	0	0	0	0	0	0	0	65	0	0
Rosa rugosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	7	1	0	0	0	0	0	0	0	38	0	0
Rosa sempervirens	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Rubus aff. wahlbergii MS-2014	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	4	0	17	0	0	0	0	0	0	0	0	6	0	0
Rubus bollei	0	0	2	3	0	2	0	0	3	0	2	0	0	0	0	3	2	1	2	2	0	0	0	0	0	2	0	8	0	0
Rubus montanus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Rubus palmensis	0	0	202	218	2	243	10	59	114	0	551	1	0	0	0	19	70	339	117	90	0	0	1	0	0	292	0	1	1	0
Rubus scissoides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	188	0	0	2	64	0	0	58	0	0	0	0	3	0	0
Rubus ulmifolius x Rubus caesius	0	0	152	178	0	82	7	14	72	0	47	0	0	0	0	0	68	25	12	38	0	0	0	0	0	94	0	1	0	0
Rumex acetosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0
Salix dunnii	0	0	0	0	19	18	12	2	221	0	0	0	0	1097	1074	0	0	0	1	0	230	0	0	40	695	0	0	0	36	0
Salix warburgii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0
Salix wilsonii	0	0	0	0	2	0	0	0	15	0	0	0	0	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0
Salvia virgata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	384	0	0	0	1	0
Sambucus nigra	0	0	0	0	0	0	241	67	1	0	0	0	0	0	0	3	1	4	4	9	0	0	0	0	0	0	0	0	1	0
Saxifraga cespitosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	0	0	0
Scandix pecten-veneris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Senecio kerdousianus	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Senecio rupestris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Silene latifolia	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sinapis arvensis	16	0	0	0	0	0	0	0	2	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Smyrnium olusatrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Solidago virgaurea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
Sorbus aucuparia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sorbus californica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sorbus chamaemespilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sorbus dumosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sorbus pseudofennica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Sorbus randaiensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Sorbus scopulina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	301	4	0	0	0	0	0	0	0	0	0	0	13	0	0	94
Stellaria media	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	48
Stellaria neglecta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	289	0	0	0	0	31
Stellaria pallida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0
Styrax officinalis	0	0	1	0	18	209	0	405	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sulla coronaria	0	0	0	0	0	0	0	0	0	0	711	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Symphoricarpos albus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Symphoricarpos orbiculatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	0	0	7	24	0	0	0	0	0	0	0	0	0	0
Syringa vulgaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0	1	0	0	0
Taraxacum (sect. Naevosa) sp. 265-2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	43	0	0	0	0	0	0	0	0	0
Taraxacum sp. Lepschi 4865	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	289	0	0	3	1	0	0	0	0	0
Tilia caroliniana	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Torminalis sp. 5752	0	0	0	0	8	0	0	71	0	0	0	0	0	0	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium berytheum	0	0	0	6	0	1	0	0	0	0	1245	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium glomeratum	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium incarnatum	0	0	0	0	2	0	0	0	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium nigrescens	0	0	0	0	0	0	8	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0

Table S6: Continued

Species	Gb1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I15	D16	D17	D19	D20	D21	D22	L23	L26	L28	L29	P31	F33	F34	G36	A37	P41
Trifolium squamosum	0	0	0	74	0	5	0	0	8	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium suffocatum	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	44	0	55	0	3	0	6	0	0	0	1	0	0	0	0
Trigonella anguina	7	0	0	0	0	73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tripleurospermum maritimum	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1868	164	0	555	0	0	0	1	0	229	0	0	0	0
Ulex europaeus	0	0	0	0	0	0	0	0	0	0	0	0	0	184	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulex gallii	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulex parviflorus	0	0	0	0	0	0	0	1	0	0	0	0	0	306	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulmus rubra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	0	0
Verbascum ifranensis	3	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
Verbascum lychnitis	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Verbascum phoeniceum	0	181	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Verbena officinalis	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronica chamaedrys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	7	0	0	0
Veronica persica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	123	0	19	0	0	0
Viburnum opulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0
Viburnum tinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0
Viburnum veitchii	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vicia faba	1	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	333	1448	548	1	0	2728	0	0	1	0	0	0	0
Vitis riparia	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xanthium saccharatum	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xanthium strumarium	0	15	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0