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Authenticity of urban honey: Specific challenges for palynology and how diversification of methods can overcome them

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Honig wohnt in jeder Blume, Freude an jedem Orte,
man muss nur, wie die Biene, sie zu finden wissen.

– Heinrich von Kleist

For my former classmates who were never able to complete their academic education because they became affected by war crimes. May the day come when we all fly free like the bees and flowers grow from the rubble.

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1. ABSTRACT

As the popularity of urban beekeeping continuously increases, there is a growing need to establish analytical criteria for the authenticity of urban honey. Across three years, this research investigates the botanical characteristics of urban honey through pollen analysis and reveals the most important foraging plants for honeybees in twelve Central European cities. Two preparation methods for light microscopy (hydrated and acetolysed) were applied to compare their advantages for melissopalynological analysis. Additionally, the role of open-access databases in supporting pollen identification was elaborated.

Urban foraging resources are defined by the distinctive ecological and socio-cultural features of cities. Pollen spectra in honey from urban apiaries show high floral diversity, shaped by the heterogeneity of managed green spaces. They strongly reflect local vegetation patterns influenced by private and municipal planting schemes. Climate-adapted urban trees are notably present in almost all samples. Late-flowering ornamental and/or invasive trees such as Ailanthus altissima and Sophora japonica extend the urban foraging season. In suburban honey, agricultural crops play only a minor role. Year-to-year variation in honey from the same apiary can be attributed to various abiotic parameters, including weather conditions.

Both the hydrated and acetolysed method are effective for the identification of common pollen types and their relative abundances. The hydrated method is more suitable in diversity studies. DNA metabarcoding may offer complementary insights.

This study provides a foundation for refining both scientific methodology and regulatory frameworks for the assessment of urban honey. It shows that light microscopy remains a relevant analysis tool. Results may serve further studies in the context of ecology climate change and honeybee foraging. To reflect the globalised and heterogeneous floral compositions typical of urban environments, traditional geographic labelling frameworks may require revision.

2. ZUSAMMENFASSUNG

Die zunehmende Beliebtheit urbaner Bienenhaltung macht auch die Etablierung neuer Analysekriterien notwendig, mit denen die Authentizität von Stadthonigen beurteilt werden kann. Die vorliegende Arbeit untersucht über einen Zeitraum von drei Jahren die botanischen Merkmale von Stadthonig mittels Pollenanalyse. Sie zeigt die wichtigsten Trachtpflanzen für Honigbienen in zwölf mitteleuropäischen Städten auf. Zwei lichtmikroskopische Präparationsmethoden (Hydratation und Acetolyse) wurden angewendet, um deren jeweilige Vorteile miteinander zu vergleichen. Darüber hinaus hebt die Studie hervor, wie wichtig frei zugängliche Referenzdatenbanken für die zuverlässige Pollenbestimmung sind.

Das Nahrungsangebot für Honigbienen im urbanen Raum ist geprägt von besonderen ökologischen und soziokulturellen Merkmalen sowie einer großen Heterogenität an Grünflächen. In der hohen Pollenvielfalt im Honig spiegelt sich die lokale Vegetation mit vielfältigen privaten und städtischen Anpflanzungen wider. Klimawandel-angepasste Ziergehölze sind in beinahe allen Honigproben vertreten. Spätblühende „Exoten“ und/oder invasive Baumarten wie der Götterbaum (*Ailanthus altissima*) oder der japanische Schnurbaum (*Sophora japonica*) verlängern den Sammelzeitraum städtischer Honigbienen. Am Stadtrand spielen landwirtschaftlich genutzte Pflanzen nur eine untergeordnete Rolle. Variationen in den Pollenspektren unterschiedlicher Jahre sind auf abiotische Parameter wie die jeweiligen Witterungsbedingungen zurückzuführen.

Sowohl die Hydratation als auch die Acetolyse eignen sich gut für die Bestimmung der Pollentypen und ihrer relativen Häufigkeiten im Honig. Die Hydratation ist insbesondere bei Diversitätsstudien vorzuziehen. Ergänzende Verfahren wie DNA-Metabarcoding können interessante zusätzliche Einblicke geben.

Diese Forschungsarbeit liefert die Basis für die Weiterentwicklung sowohl der wissenschaftlichen Methodik als auch der regulatorischen Rahmenbedingungen für die Bewertung von Stadthonigen. Sie zeigt, dass die Lichtmikroskopie weiterhin ein

relevantes Instrument für melissopalynologische Untersuchungen ist. Die Ergebnisse können als Grundlage für weiterführende Studien im Kontext von Ökologie, Klimawandel und dem Sammelverhalten von Honigbienen dienen. Konventionelle Herkunftsanalysen sind so zu überarbeiten, dass sie den Besonderheiten von Stadthonigen flexibel gegenüberstehen und das für Städte typische globalisierte, heterogene Trachtangebot widerspiegeln.

3. GENERAL INTRODUCTION

3.1. BIOLOGICAL AND AGRICULTURAL SIGNIFICANCE OF HONEYBEES

Honeybees play a vital role for the ecosystem. They offer pollination services to plants by collecting nectar and pollen from flowers. In return, receive nutrients which they store as honey. Beekeeping has enabled humans to harvest honey to enrich their diet and trade it as a valuable commodity.

3.1.1. The species *Apis mellifera*

The Western honeybee, *Apis mellifera*, is a eusocial, state-building Hymenoptera species native to Europe. Colonies consist of a single fertile “queen”, up to 50.000 non-fertile female “worker bees”, and a few hundred male “drones”. They communicate and organise themselves through complex patterns of dance language as well as pheromones (Seeley 1985; Crane 1990; Johnson 2023). Foragers collect nectar and other plant/insect excretions with their proboscis, store it in a separate honey stomach, and regurgitate it upon arriving back in the hive (Goodman and Munn 2022). Other worker bees add enzymes like invertase, which breaks down sucrose into the simple sugars glucose and fructose. It is then stored in beeswax cells in the honeycomb, where evaporation causes the water content to decrease greatly and form the viscous substance known as honey (Crane 1990; Moosbeckhofer and Bretschko 1996; Johnson 2023). The typical flight radius of forager bees is 2-3 km, although scout bees may explore resources as far as of 6-10 km from the hive (Couvillon et al. 2015; Goodman and Munn 2022).

Out of the genus *Apis*, only two species have been truly cultivated: *Apis mellifera* and *Apis cerana*, which is restricted to Asia. Europe is native to several subspecies of *Apis mellifera*. The Carniolan honeybee, *A. m. carnica*, originates in the Carpathian plains, Slovenia and Southern Austria. It is known to behave gently and to be a highly productive honey producer (Ruttner 1988; Sulzner 2016; Moškrič et al. 2022). The European dark bee, *A. m. mellifera*, is widely distributed from the Ural Mountains in Russia to the Pyrenees in Spain, including Scandinavia, and the British Isles (Crane 1999). It is better adapted to colder climates and shows more defensive behaviour (Uzunov et al. 2014). In Austria, it overlaps with the distribution area of

A. m. carnica (Ruttner 1988; Moosbeckhofer 2007). Other subspecies used for commercial beekeeping in Central Europe are *A. m. ligustica* and the Buckfast bee. While the Ligustica bee is native to Italy, the Buckfast bee emerged from a breed of *A. m. mellifera* and *A. m. ligustica* (Faugere and Dussy 2023). All subspecies display a generalist foraging behaviour. According to Köppler (2007), the spectra of collected pollen differs in date of sampling and sampling location, but not between various *Apis mellifera* subspecies. Honey analysed for this research was solely collected by *A. m. carnica* bees, which is the most commonly kept honeybee breed by commercial and private apiarists in Austria and the surrounding areas (Moosbeckhofer and Bretschko 1996; Moosbeckhofer 2007).

3.1.2. Honeybee nutrition

Both larvae and adult honeybees obtain their nutrients from plants. Their main source for carbohydrates is nectar and their main source for proteins and lipids is pollen. The long-term storage of nectar is honey, while pollen is stored in beebread (perga) for later consumption (Crane 1990).

Not all nectar and pollen sources are of equal value for the honeybee diet. Nectar composition can vary greatly between plant taxa, with a sugar content typically ranging between 20% and 40% (Brodschneider and Crailsheim 2010). The main sugars comprising nectar are fructose, glucose, and sucrose, with higher molecular sugars such as maltose, trehalose and melezitose also present. The protein content of pollen is also species-dependent and lies between 10% to 30% (Liolios et al. 2015) or even 60% (Wright et al. 2018). Honeybees need multiple floral sources to manage their need for essential amino acids like leucine, isoleucine, and valine (Brodschneider and Crailsheim 2010; Liolios et al. 2015). However, when facing protein shortages, colonies seem to adjust the rate of pollen collection rather than going for higher quality pollen types (Pernal and Currie 2001). Pollen grains also contain 7% to 20% lipids, the most important essential fatty acids being alpha-linolenic acid and linolenic acid. Vitamins and minerals are usually foraged along with water but are also contained in nectar and pollen (Johnson 2023). Optimal nutrition is best achieved through foraging on different plant taxa in a diverse environment (Roulston and Cane 2000; Liolios et al. 2015).

3.1.3. From honey hunting to modern apiculture

The history of honey harvesting dates back at least 4,500 years and has for the most part been a process of hunting for wild hives (Crane 1999). After the domestication of honeybee colonies, beekeeping turned into a profession. Improved hive design with moveable frames revolutionised beekeeping in the 19th century and simplified hive management, pest control, and honey harvesting (Moosbeckhofer and Bretschko 1996; Crane 1999). While this accelerated the commercialisation and professionalism of apiculture, the structure of beekeeping in Austria remains small-scaled with an average of 13 hives per enterprise and only 1.5% professional apiarists (Boigenzahn 2025). Honey extraction times are closely tied to the seasonal rhythm of honeybees, which is coupled with the progression of climate and plant phenology (Seeley 1985). Depending on the environment and the primary nectar sources, many beekeepers in Central Europe are able to extract twice within the vegetation period: A first time in spring (May–June) and a second time in summer (July–August). In areas with less abundant floral resources, a single harvest in June–July is common (Moosbeckhofer and Bretschko 1996). The actual honey yield per colony may vary widely according to the climatic region, the *Apis mellifera* subspecies, and supplementation with carbohydrates (Brodschneider and Crailsheim 2010).

3.2. HONEY AS A FOOD PRODUCT

Until the establishment of industrial sugar production in Europe around the year 1800, honey was the only known sweetener. Apart from sugars and water, honey also contains organic acids, minerals, vitamins, and enzymes. Through its antimicrobial and probiotic properties, honey offers additional health benefits (Crane 1999). Today, honey is marketed in a multitude of packaged and semi-processed ways, as well as being a major ingredient in snacks, baked goods, and cereals (Bellik and Iguer-Ouada 2013). It has become a multi-billion-dollar business and globally traded commodity with well established distribution lines. As such, authenticity, adulteration, and consumer safety are constant issues, requiring strict regulations on what can be sold and under which label (EC 2002; Soares et al. 2017; Thrasyvoulou et al. 2018; AGES 2020).

3.2.1. Global honey market and consumption

The average honey consumption per capita in the European Union ranges from 0.3–0.4 kg in France and Great Britain to 1.0–1.8 kg in Germany and Austria (Bellik and Iguer-Ouada 2013). Economically, the biggest producers of honey worldwide are China, Turkey, Ethiopia, Iran, Argentina, India, Russia, Brazil, United States, and Mexico (FAOSTAT 2025). Honey production in the European Union marked 285,700 tons in 2022. An additional 363,077 tons were imported, while 17,533 tons were exported from the EU (CBI 2024). The global honey market size is estimated at USD 9.45 billion in 2023 (Grand View Research 2025).

3.2.2. Honey authenticity and testing

According to the Codex Alimentarius, honey is “the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature” (Codex Alimentarius Commission 2022).

Honey as a food product is particularly prone to adulteration, because of the high market demand and limited natural production by honeybees. Testing for authenticity is necessary to ensure quality standards such as the nutritional value of honey, consumer safety, and correct labelling (Bogdanov and Gallmann 2008; Soares et al. 2017). This is typically done at specialised laboratories and follows national and international legislation (Thrasyvoulou et al. 2018). Such tests consist of sensory, physico-chemical, and melissopalynological components. At first, the taste and smell of a sample is compared to the label under which it is distributed (Marcazzan et al. 2018). Physico-chemical testing includes moisture content, sugar profile, electrical conductivity, enzyme activity, free acidity, and the pH level (Persano Oddo and Piro 2004). Botanical and geographical origin is examined with the help of pollen analysis (Louveaux et al. 1978; Von der Ohe et al. 2004; Soares et al. 2017). As a natural product, honey does not contain any additives or preservatives. Further, to be sold as a food product, it must not be filtered, heated, or have started to ferment (EC 2002).

Honey fraud can be divided into two main categories: 1. The production of honey, which covers the filtration of pollen, thermal treatment, increased water content, and the addition of sugar syrups; 2. Honey labelling concerns both the botanical and geographical origin as well as, e.g., “organic” beekeeping practices (EC 2002; Bogdanov and Gallmann 2008; Soares et al. 2017). The relation between analysis methods and regulative legislations on the one hand and adulteration practises on the other hand can be described as “coevolution”. Ever new methods of fraud require a constant update of control mechanisms. Melissopalynology may not be suitable for the detection of all fraudulent practises, such as the filtering and the addition of customised pollen compositions. It does however provide good identification success on the level of plant species/genera and thus is still invaluable for the analysis of foraged plants and geographical origin (Soares et al. 2017).

Honey labelling differentiates between multifloral and unifloral honey as well as honeydew honey:

3.2.2.1. Multifloral honey

Multifloral (polyfloral) honey is defined as wholly or mainly from nectar of flowers and extrafloral nectaries. It has been foraged by honeybees from a multitude of floral resources. Colour, viscosity, and flavour profile can vary, depending on the vegetation surrounding the hive. Organoleptic, physico-chemical, and melissopalynological characteristics do not meet the criteria for unifloral honey. The jars therefore must be labelled in a more general sense as ‘multifloral honey’ and/or according to the seasons in which they were harvested in (EC 2002; AGES 2020).

3.2.2.2. Unifloral honey

Unifloral (monofloral) honey has the predominant character of a single floral source. This plant species is responsible for the unique flavour, aroma, colour, and viscidity of the honey (Persano Oddo and Piro 2004). Multiple tests have to be passed before the respective honey can be sold as unifloral. This includes organoleptic and physico-chemical evaluation as well as the analysis of botanical origin through pollen analysis (Beckh and Camps 2009). Whether a plant species gives unifloral honey depends on several factors, including the amount of nectar production, a distinct flowering period, honeybee foraging behaviour, and the plant diversity in the

vicinity of the hive. Of the more than 100 taxa giving unifloral honey in Europe, some are widely distributed and relevant for commercial reasons, while others are produced only on a local scale (Persano Oddo et al. 2004). Typically, unifloral honey is sold at a higher price than honey blends (Persano Oddo & Bogdanov 2004). The main plant species contributing to unifloral honey in Central Europe are highlighted in Table 1. Percentage thresholds may vary according to national legislation (Thrasyvoulou et al. 2018).

Table 1: Main botanical species giving unifloral honey in Central Europe, as well as the required range of percentages (relative abundances of particular pollen species in the honey).

English Name	Deutscher Name	Latin name	Range	Reference
Oilseed Rape	Raps	*Brassica napus*	60-99%	Persano & Piro 2004
Heather	Besenheide	*Calluna vulgaris*	10-77%	Persano & Piro 2004
Sweet Chestnut	Edelkastanie	*Castanea sativa*	86-100%	Persano & Piro 2004
Cornflower	Kornblume	*Cyanus seggetum*	20% (> 8%)	Beckh & Camps 2009
Erica (heather)	Erika	*Erica* sp.	> 45%	Beckh & Camps 2009
Buckwheat	Buchweizen	*Fagopyrum esculentum*	30%	Beckh & Camps 2009
Fennel	Fenchel	*Foeniculum vulgare*	68% (> 55%)	von der Ohe 2016
Sunflower	Sonnenblume	*Helianthus annuus*	12-92%	Persano & Piro 2004
Lavender	Lavendel	*Lavandula* sp.	1-20%	Persano & Piro 2004
Stone fruits	Steinobst	*Prunus sp.*	68% (> 60%)	Beckh & Camps 2009
Alpine rose	Alpenrose	*Rhododendron* sp.	3-20%	Persano & Piro 2004
Black locust	Robinie (Akazie)	*Robinia pseudoacacia*	7-60%	Persano & Piro 2004
Raspberry	Himbeere	*Rubus* sp.	82% (>71%)	Beckh & Camps 2009
Dandelion	Löwenzahn	*Taraxacum* sp.	5-41%	Persano & Piro 2004
Linden (Lime tree)	Linde	*Tilia* sp.	1-56%	Persano & Piro 2004
Clover	Klee	*Trifolium* sp.	70% (> 60%)	Beckh & Camps 2009

3.2.2.3. Honeydew honey

Honeydew honey consists partly or mainly of excretion products from phloem feeders such as certain aphids (Ali et al. 2024). Typical botanical origins of honeydew are *Abies* sp. (fir), *Picea* sp. (spruce), and *Pinus* sp. (pine tree). Deciduous tree species giving honeydew are, e.g., *Quercus* sp. and *Tilia* sp. (Heimbach 1986). However, the label “honeydew honey” or “Waldhonig”, is restricted to honey from forested areas. Honey from urban parks and green spaces must not be labelled as such (DLMBK 2023). From a physico-chemical perspective, honeydew honeys typically have a higher pH value and electric conductivity as well

as a different sugar spectrum than multifloral or unifloral honey (DLMBK 2023). Microscopical characteristics of honeydew honey are fungal spores, microalgae, and wax elements.

3.2.2.4. Other bee products

Other bee products, which are commonly harvested and sold for human consumption, include corbicular pollen pellets, propolis, and royal jelly (Crane 1999). Pollen pellets are collected off the hind legs of the forager bees with specialised combs at the entrance of the hive. They are of different colours, depending on their predominant floral source (Kirk 2018). Propolis is a resinous mixture of beeswax, phloem exudates, and honeybee saliva. It is produced by bees to construct, seal, and repair the hive. Thanks to its antimicrobial properties, it is used to treat illnesses such as cough and wound healing (Walker and Crane 1987; Crane 1999). Royal jelly is the nutrient mixture secreted by nurse bees to feed the queen larvae. It is high in proteins, mineral content, and vitamins (Crane 1999; Johnson 2023).

3.3. URBAN HONEY

3.3.1. Characteristics of the urban environment

The degree of urbanisation has increased continuously over the last decades. With cities spreading and becoming more dense, natural landscapes cease. Species have to adapt to urban conditions, including an altered microclimate, habitat fragmentation, and a new array of food sources and competitors (Goulson et al. 2015). Plant species are introduced and maintained for aesthetic reasons, which favour some pollinators and endanger others (Bates et al. 2011; Baldock 2020; Prado et al. 2024).

3.3.1.1. Climate, green spaces, and intentional planting

The climate in cities may differ drastically compared to the surrounding rural areas, due to the density of built elements and impermeably sealed surfaces. Anthropogenic emissions of light, heat, and air pollutants interfere with atmospheric processes like radiation and evaporation, leading to the so-called urban heat island effect (Oke 2017; Marando et al. 2022). Urban green spaces may consist of parks, private gardens, cemeteries, road verges, rail tracks, industrial sites, brown fields,

green rooftops and facades, allotments, urban farms, and remnant natural vegetation (Lepczyk et al. 2017; Baldock et al. 2019; Ayers and Rehan 2021). The quality of different types of green spaces can be described as continuum from original plant communities to highly altered patches of ornamental and invasive plants, requiring a varying degree of long-term maintenance (Bates et al. 2011; Baldock 2020; Bell et al. 2023). The higher the individual patch size and connectivity between isolated green spaces, the higher is their positive impact on the natural ecosystem (Lepczyk et al. 2017). Plants, however, need to be well adapted to heat and drought when growing in urban areas. This may favour non-native plants (Sjöman et al. 2012; Sponsler et al. 2020; Fox et al. 2022). Additionally, urban green spaces are often shaped by intentional choice of species or compositions planted by private and municipal gardeners.

3.3.1.2. Insect pollinators in cities

The composition of insect species is filtered by their ability to deal with the unique features of urban landscapes. Many thermophilic species prefer the warm and dry condition of inner-city environments over those in seminatural or rural areas (Hall et al. 2017; Baldock 2020). However, through habitat modification and the urban heat island effect, a simplification of functional trait diversity in urban wild bee communities was reported (Baldock 2020; Ferrari and Polidori 2022). Conditions in densely built-up areas require sufficient flight ability to deal with habitat fragmentation, less strict dietary specialisation, and the availability to find nesting sites. Insect pollinators in cities are more polylectic in their diet, depict a higher cavity nesting behaviour, and later emergence (Bates et al. 2011; Ayers and Rehan 2021). On the other hand, specialist foragers are underrepresented because of the various qualities of green spaces and their loose connectivity (Bates et al. 2011; Lepczyk et al. 2017). In general, there seems to be an incongruence between what plants urban bees and urban gardeners find attractive (Bell et al. 2023). However, the willingness among garden centre customers to preferentially choose bee-friendly plants is great (Wignall et al. 2019).

3.3.2. Urban beekeeping

Beekeeping has gained popularity across many urban areas worldwide. The density of honeybee colonies in some Central European cities by far exceeds the one in the

countryside (Casanelles-Abella and Moretti 2022). In Vienna, around 5,000 honeybee colonies are kept by 700 apiarists (Stadt Wien 2025a), which equals 12 hives per square kilometre. With around 4,500 beehives within its municipal borders, the density in Ljubljana is even more than twice that of Vienna (City of Ljubljana 2025).

There are multiple socio-ecological benefits to urban beekeeping (Egerer and Kowarik 2020; Sponsler and Bratman 2021): As regionality and transparency are in high demand among customers, it can be a source of local supply parallel to urban gardening. When run in a sustainable way, urban apiaries can be a chance to teach city dwellers about the environment and importance of pollinator conservation (Cho and Lee 2018). They create livelihoods by providing apiarists with economic opportunities. Urban honey can be offered at elevated prices, with eye-catching labels and the potential to generate additional benefits from advertisement of a certain landmark or rooftop (Soares et al. 2017). Urban apiaries also bring together an expert community of beekeepers, urban planners, and scientists. On the other hand, there is some concern about the competitive stress on wild bees (Ropars et al. 2019; Weissmann et al. 2021; Gratzer and Brodschneider 2023), the transmission of diseases to wild pollinator assemblages, and stinging (Sponsler and Bratman 2021; Matsuzawa and Kohsaka 2022; Melathopoulos et al. 2025). Colla (2022) uses the term “bee-washing” for the negative impact of the urban beekeeping hype on wild bee conservation efforts, criticising misleading information and misallocation of resources.

The extent of urban beekeeping largely depends on local regulations and governing regimes. Some cities offer a high amount of support, e.g., through advice for nuisance-free residential beekeeping, offering training and services for apiarists, research grants, and consideration in urban green planning (Melathopoulos et al. 2025; Erhart et al. 2022). Other cities may have restrictions in place or are planning on establish them, or they limit the number of apiaries due to human safety and/or ecological concerns (Casanelles-Abella and Moretti 2022; Matsuzawa and Kohsaka 2022). In Austria, restrictions primarily concern pest control and the distance to the neighbours’ property lines (Wiener Landtag 2000).

3.3.3. Specific challenges for authenticity of urban honey

The urban beekeeping boom comes along with strong marketing efforts for a variety of honeybee products (honey, corbicular pollen loads, or value added derivates like oxymel and mead), as well as seasonal beehive renting and management services for businesses and private individuals (Sponsler and Bratman 2021). Major concerns for authenticity of urban honey include geographical origin and incorrect labelling. From a food safety perspective, urban pollutants may create potential health hazards.

3.3.3.1. Origin and labelling

Inner city rooftops and private gardens can typically only host a small number of honeybee colonies. The net weight of urban hives in a study in Latvia increased continuously throughout summer, rather than in peak-patterns related to the flowering of mass crops in spring (Komasilova et al. 2023). This may shift harvesting times and limit the honey material from a one city site to often not more than 100 kg a year, which is in stark contrast to the high demand of honey from, e.g., a certain address or landmark. Transparency can be vague in order to disguise potential blending practises (Soares et al. 2017) and labelling can be intentionally misleading. In addition, regulative methods relying on pollen analysis may be insufficiently acknowledging the altered plant communities in urban areas with high amounts of non-native plants (Bates et al. 2011; Sponsler et al. 2020; Fox et al. 2022; Bell et al. 2023).

Current legislature (EC 2002; AGES 2020; DLMBK 2023) offers guidelines to honey labelling in the general terms. So far, they have indifferently been applied to honey from inner city apiaries. However, most commercial urban honey distributers use labelling far more detailed and specific regarding a certain site. According to (Soares et al. 2017), labelling honey in such detailed manner requires to proof that the exact pollen type combination is unique to this location and cannot be expected in the surrounding areas. However, because of the typical 3 km flight radius of honeybees (Couvillon et al. 2015), a differentiation between neighbouring political districts or even rooftops is purely speculative (Soares et al. 2017) and as such alone is not compliant with regulations on the labelling of geographical origin (AGES 2020).

However, next to all other national and international labelling requirements, it is not illegal to add geographical references to the label for marketing purposes.

3.3.3.2. Food safety and contaminants

Several studies have researched the safety of honey for consumers in regards to heavy metal contamination, the presence of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), pollen from GMO plants, antibiotics and pesticides (Soares et al. 2017; Quiralte et al. 2023). Potential contaminations are either due to environmental contamination of nectar and pollen, or due to treating hives in the apiary (Bogdanov 2006; Passarella et al. 2022). The level of heavy metals was generally attributed to air pollution (Jovetić et al. 2018) and the elevated levels of the PAH naphthalene to combustion (Passarella et al. 2022). The seasonal differences of PAHs and PCBs concentrations in urban honeybee and pollen samples are due to fluxes in anthropogenic emissions (Sari et al. 2020). As matter of food safety, Jovetić et al. (2018) reported that all their analysed honey samples from Belgrade, Serbia, met the international and national quality criteria for safe human consumption. There are still no established maximum levels of PAHs, PCBs, heavy elements, and other contaminants in honey (EC 2010; Thrasyvoulou et al. 2018; Quiralte et al. 2023).

3.4. PALYNOLOGY

3.4.1. Pollen development

Pollen grains are the reduced gametophyte of seed plants and contain the male gametes (sperm cells). They only possess one set of chromosomes, since they develop through meiosis of pollen mother cells in the microsporangia of anthers (angiosperms) or cones (gymnosperms).

During microsporogenesis, diploid microsporocytes are enclosed by a thick callose layer and undergo meiosis, in which a tetrad of haploid microspores is formed. Polarity as well as aperture orientation of the pollen grain are determined at this stage (Figure 1, from Halbritter et al. 2018). Each of these microspores is then itself enclosed with callose and develops into a fertile pollen grain through the process of microgametogenesis. In a first mitotic division, a large vegetative cell with vegetative

nucleus and a much smaller, spindle-shaped generative cell are formed. The second pollen mitosis produces two sperm cells. This happens either still in the anther, or later in the pollen tube after successful pollination. Whether pollen grains are dispersed in a 2-celled or 3-celled stage depends on the plant taxon (Brewbaker 1967; Williams et al. 2014).

During the maturation of pollen grains, the exine and the intine layer of the pollen wall are formed. At first, primexine is deposited on the surface of the microspores. The endoplasmic reticulum of the microspore cell inhibits the deposition of primexine in the predetermined aperture positions. Later, the primexine template is substituted by sporopollenin to build the final pollen wall. A layer of specialised tapetum cells around the microspore is responsible for its nourishment as well as the synthesis of the callase enzyme, exine precursors, Ubisch bodies, viscin threads and pollenkitt (Halbritter et al. 2018). Before anthesis, the mature pollen grain dehydrates, which preserves it for dispersal. The mature anthers dehisce and release the pollen.

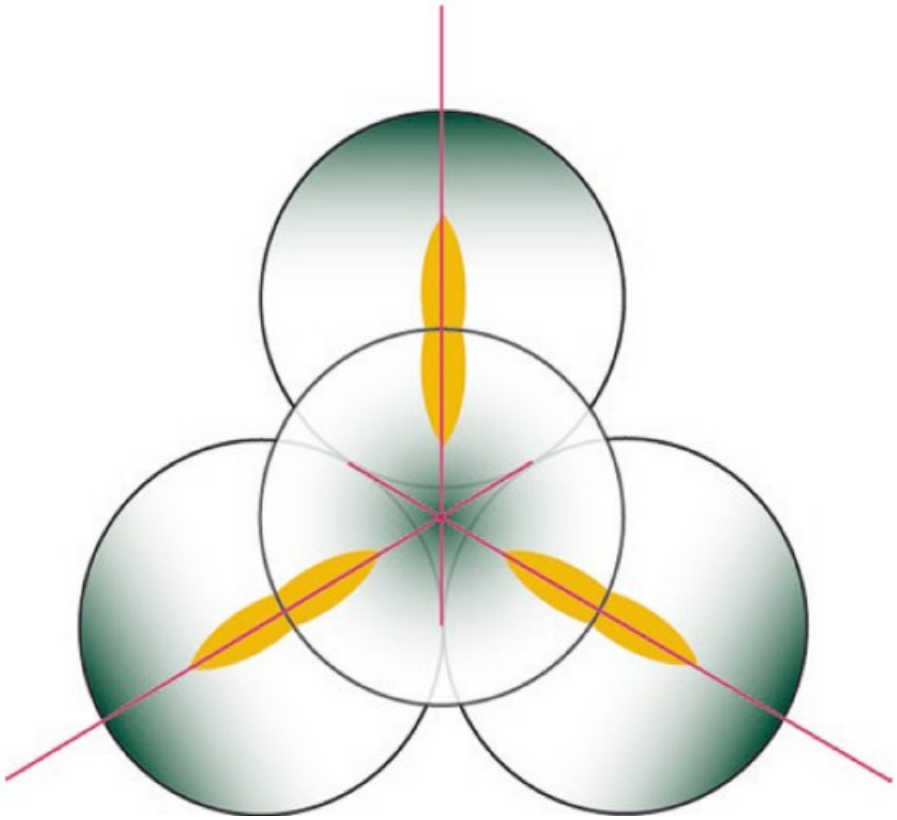

Figure 1: Configuration of microspores and tetrad pollen grains with the apertures (yellow), polar axis (red line), and distal poles (shaded green). In: Halbritter et al. 2018, p.38.

3.4.2. Pollination mechanisms

Dispersal of pollen grains is vital for the reproduction and distribution as species. Plant taxa have developed various strategies for short- and long-distance pollen dispersal, which involve biotic or abiotic vectors. The most common are:

- Anemophily (wind)
- Zoophily (animals)
- Hydrophily (water)
- Autophily (self-pollination)

Wind pollinated plants typically produce masses of pollen, which is exposed on reproductive structures such as cones or spikes. Anemophily is ideal for long-range transport and the predominant pollination syndrome in higher latitudes. Pollen, e.g., from pine trees, can travel thousands of kilometres by wind (Willmer 2011).

The majority of animal pollinators are insects (entomophily), bats (chiropterophily), and birds (pterophily), although the latter two occur mostly in the tropics (Willmer 2011). Insect pollination is limited to the flight range of pollinators. Plant taxa have evolved distinct pollination syndromes to attract specific pollinators. They depict certain colours, shapes, and odours, oftentimes in combination with nectar guides (e.g., Asteraceae). In a mutualistic relationship, plants foraged by honeybees and other hymenoptera typically display nectar and pollen as reward for pollinators. Certain bee species also collect oils, resins, and scents (Willmer 2011). Much more rarely, hydrophily is the transportation of pollen by water, which usually happens on the water surface (Taylor et al. 2020).

Self-pollination in plants can be divided into autogamy, in which the pollen of the anther pollinates the stigma in the same flower (e.g., many kinds of orchids), and geitonogamy, in which the pollen is transferred to another flower or microsporangium of the same plant individuum. Cleistogamy is a specialised form of autogamy, where flowers do not fully open, such as in *Viola* species (Ammarellou et al. 2021).

3.4.3. Pollen morphology

Pollen morphology is described through different parameters in relation to the structure and form of pollen grains. Morphological identification of pollen grains is important in order to understand taxonomic relations, evolutionary biology, ecology, and plant reproductive biology. The main parameters are:

- Dispersal unit
- Shape and symmetry
- Size (length of polar axis and diameter)
- Number, shape, and arrangement of apertures
- Ornamentation of the pollen wall

The shape of pollen grains is often determined by the dispersal mechanism. Anemophilous pollen does usually not have prominent sculptural elements or pollenkitt. Some gymnosperms such as Pinaceae are saccate. Pollen grains of zoophilous plant taxa have a rougher surface, oftentimes with sculptural elements such as striae or echini. Pollenkitt, a gluey substance produced in the anther, may be present for adherence. Pollen transported in or on the water typically has a very thin wall and reduced apertures (Punt 1986; Pacini and Hesse 2005).

The main functions of the aperture are harmomegathy, ion exchange, and germination (Thanikaimoni 1986). In order to protect the cytoplasm from dehydration, plants may reduce the aperture area. For germination on the flower, pollen grains are rehydrated in liquid on the stigma of flowers. The larger the aperture area and number, the quicker the pollen tube is formed and can transfer the sperm cells to the gynoecium. Factors influencing the exine pattern of pollen include transportation, protection, germination, as well as genetic factors (Punt 1986). The pollen wall can be very thick in species that rely on pollination by larger insects, bats, and birds.

3.4.4. Pollen ultrastructure

The term ultrastructure is used for cellular and sub-cellular components of the pollen wall and the cytoplast. The two primary layers of the angiosperm pollen wall are the

intine and the exine. Subclassifications, additional layers, or reductions can occur depending on the plant taxa and its evolutionary relations (Figure 2, from Halbritter et al. 2018). The use of transmission electron microscopy (TEM) provides a detailed view of the internal wall construction. The exine structure is comprised of the complex and very durable biopolymer sporopollenin (Straka 1975; Halbritter et al. 2018), which is often naturally coloured or artificially stained and used for pollen identification in LM. The intine, endexine and foot layer may appear in the LM as a gap between exine and cytoplast.

Some of the contents of the cytoplast can be observed with the LM as well. To determine whether a pollen type is binucleate or trinucleate (Brewbaker 1967), a drop of acetocarmine is added to fresh pollen. The nuclei of the two sperm cells are usually more spindle-shaped and stain darker than the generative nucleus (Halbritter et al. 2018). If starch is present, potassium iodine will stain the starch granules dark brown. Starch reserves are disproportionally more prevalent in anemophilous species (Roulston and Cane 2000).

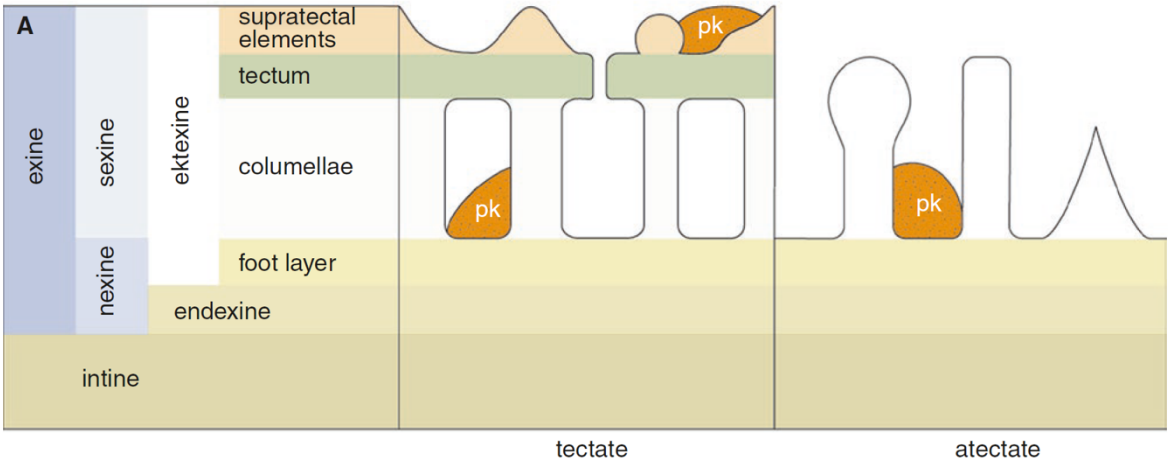

Figure 2: Structure of the pollen wall. In: Halbritter et al. 2018, p. 47.

3.4.5. Applied palynology

Various disciplines can profit from the study of pollen grains and spores. Aerobiology identifies pollen and monitors their concentration in the air to determine air quality and the burden for allergy sufferers. The latter stresses the relevance of pollen in the medical field (Dirr et al. 2023; Bastl et al. 2023). In archaeology, pollen may be

analysed from waste dumps, coprolites, and kitchen vessels in order to reconstruct ancient lifestyles, foods, medicines, agricultural habits, and environmental conditions (Weber et al. 2020; Jakobitsch et al. 2023). Forensic palynology relates identified pollen grains to potential environmental sources, timespans, and origins of the material. It involves the analysis of crime scenes, clothes, and body parts (Mildenhall et al. 2006). Palaeoecology and climate reconstruction use drill cores of sediment rocks or sediments in water bodies to assess changes in pollen spectra over long time periods (Straka 1975). Prehistoric plant-pollinator interactions can be assumed from relating fossil pollen grains to pollen on fossil insects (Geier et al. 2024).

3.5. MELISSOPALYNOLOGICAL METHODS

Melissopalynology is the study of analysing pollen grains found in honey. It is performed to examine the floral as well as the geographical origin of honey and thus ensures the quality and authenticity of honey as a food product (Von der Ohe et al. 2004; DLMBK 2023). Ecologically, melissopalynology allows to identify the types of plants that bees forage on. This may serve as an environmental monitoring tool to assess the quality and plant diversity of the surrounding ecosystem.

3.5.1. History of melissopalynology

The advance of light microscopes with sufficient magnification was the starting point of melissopalynological studies around the beginning of the 20th century. In the 1930's an increased interest in honey analyses lead to the first extensive literature works (e.g., Armbruster and Oenike 1929). Mid-20th century, Gunnar Erdtman developed the acetolysis technique for pollen (Erdtman 1943). In the last third of the 20th century, great advances in melissopalynology have been published (Louveaux et al. 1978; Sawyer 1988; Vorwohl 1995). The international standardisation of techniques for the analysis of botanical and geographical origin of honey as a food source has been attempted by members of the International Honey Commision (e.g., Persano Oddo et al. 2004; Von der Ohe et al. 2004). In more recent years, molecular methods such as DNA metabarcoding (Bell et al. 2016; De Vere et al. 2017; Fox et al. 2022) and Near Infrared Spectroscopy (Escuredo et al. 2021; Bodor et al. 2021) have been elaborated.

3.5.2. Standardised methods

3.5.2.1. Hydrated pollen

The hydrated method of melissopalynology is described in detail in the publication “Comparing the acetolysed and hydrated methods for the pollen analysis of honey” (see Chapter 4.B.). A graphic description of the preparation process can be found in the Appendix (Figure A6).

3.5.2.2. Acetolysed pollen

The acetolysed method of melissopalynology is described in detail in the publication “Comparing the acetolysed and hydrated methods for the pollen analysis of honey” (see Chapter 4.B.). A graphic description of the preparation process can be found in the Appendix (Figure A7–A8).

3.5.3. Identification and Counting

Pollen types were identified with the help of relevant literature, if available. This included online pollen databases (PalDat 2025; Stebler 2025), as well as books (Reille 1992; Bucher 2004; Beug 2021).

Counting a statistically relevant number of pollen grains is done to determine the relative abundances of identified pollen taxa. The minimum required number of counted grains depends on the type of sample and the research aims (Djamali and Cilleros 2020; Weng and Hooghiemstra 2024). In some disciplines, e.g., forensic palynology, counting out 300 individual pollen grains is usually sufficient (Weber et al. 2020). In case one taxon dominates massively, the other taxa may be counted up to 300, or 500, respectively (Weber et al. 2020). The standardised method of melissopalynology (Von der Ohe et al. 2004) requires 500 grains as the minimum value. This is due to the high diversity of pollen types in honey and the need to unify the results for official food safety analyses, in which unifloral honey is defined by percentages of the respective pollen type (Von der Ohe et al. 2004; Lau et al. 2018; Weng and Hooghiemstra 2024).

3.5.3.1. Anemophilous pollen taxa

Pollen from wind-pollinated plants is present in almost all honey samples to some extent. This is typically due to airborne contamination (Louveaux et al. 1978; Bryant

and Jones 2001). Pound et al. (2023) could show that elevated concentrations of *Pinus* pollen are correlated to large amounts of pollen in the atmosphere. Pollen of Poaceae and *Plantago*, in contrast, is oftentimes foraged intentionally. While lots of pollen analysts are ignoring anemophilous pollen from counting to 500 (Von der Ohe et al. 2004), others are advising to include them in the counts as well. This links pollinators and their resources in a diverse landscape. Ecologically, it might also point to behavioural traits of a colony and/or nutritional shortages (Saunders 2018). Honeybees in urban areas (Pound et al. 2023) and alpine environments were found to intentionally forage Poaceae pollen in higher amounts (Brodschneider et al. 2019).

3.5.3.2. Over- and underrepresented pollen in honey

The nectar:pollen ratio in a flower is responsible for the under- or overrepresentation of botanical species in honey and may lead to false conclusion in regards to the overall importance of foraging plants. In *Robinia pseudoacacia,* the ratio is very high, and therefore, *Robinia* pollen will likely not be very abundant and the threshold for unifloral *Robinia* honey is as low as 7% (Persano Oddo and Piro 2004). Other taxa underrepresented in honey include *Citrus*, *Lavandula*, and *Coriander*. *Castanea sativa*, on the other hand, produces vast amounts of small pollen grains in addition to having nectaries at the floral bases. In 10 g of unifloral *Castanea* honey, there can be up to 250,000 *Castanea* pollen grains. Hence, pollen of *Castanea sativa* is overrepresented. Other overrepresented taxa include *Myosotis* sp., *Mimosa* sp., and *Eucalyptus* sp. (Escriche et al. 2023).

Additionally, the quantities of pollen that make it into honey do not necessarily correlate to the amount of nectar which has been foraged by the honeybee from that plant species. This depends on many factors including the pollen size, time of the day, and distance between the foraging source and the hive (Bryant and Jones 2001).

3.5.3.3. Pollen coefficients

The relative percentage of a certain pollen type in comparison to its absolute pollen count per volume (APC) indicates the quantitative contribution of each plant taxon to the sample. The average number of pollen grains per gram honey is known as

pollen coefficient and was researched in controlled experiments, e.g., by (Demianowicz 1964). These coefficients are constant for each unifloral honey type and include information whether a pollen type is usually over- or underrepresented in honey. While *Castanea sativa* is largely overrepresented with a pollen coefficient of 1000/g, *Tilia* sp. produces much higher quantities of nectar than pollen and thus its pollen is underrepresented with a pollen coefficient of only 10/g (Sawyer 1988). However, due to the complexity of determining pollen coefficients for each plant species and the lack of unification, the standardised method of melissopalynology as in Von der Ohe et al. (2004) does not include total quantitative analyses with coefficients. The co-occurrence of under- and overrepresented pollen can be challenging for the identification of unifloral honey (Russo Almeida et al. 2024)

3.5.3.4. Counting tools and visualisation

For this research, a virtual pollen analysis tool was used for the counting of 500 pollen grains, as well as a visual presentation of the results. The counting tool designed by the AGES Department of Apiculture and Bee Protection was combined in Excel with the automated colour-coded diagram function used by Martina Weber in forensic palynology (Figure 3). Colours were chosen to indicate distinct plant families.

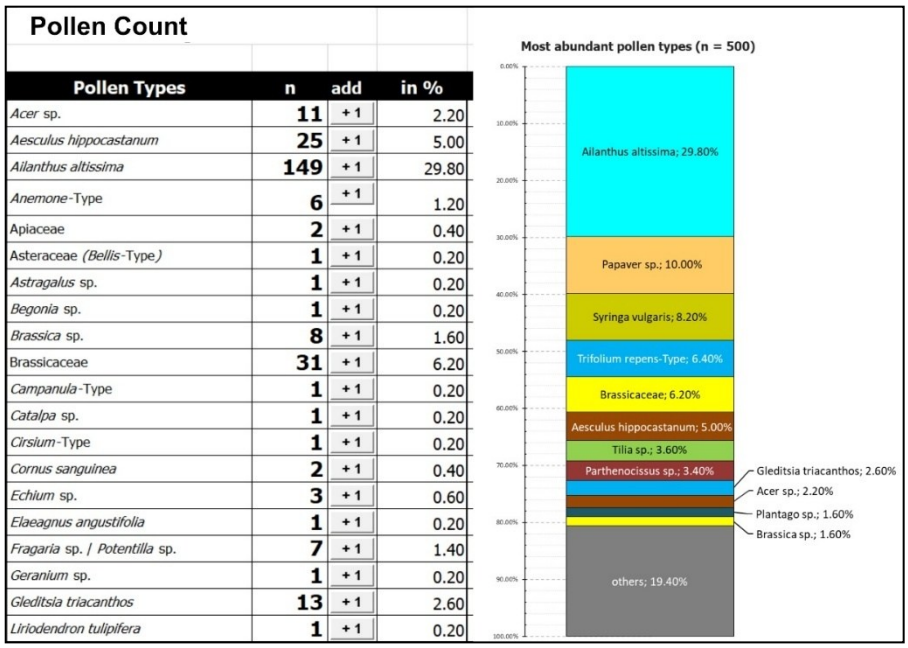

Figure 3: Virtual pollen counting tool: List of identified pollen types, macro buttons (+1) to add a pollen record (n), automatically produced relative percentages, and automatically created colour-coded diagrams of the most abundant pollen types.

3.5.4. Additional methods

Light microscopic techniques (hydrated and acetolysed) are applied in the routine analysis of honey and are suitable to examine the floral resources of honeybees. This is the only standardised method in which results from multiple decades and world regions are comparable qualitatively as well as quantitatively. Nonetheless, the abilities to detect fraud with LM are limited (Soares et al. 2017). Adulterations may concern the botanical and geographical source of honey and all sorts of contaminations. Method diversity can lead to improved results. While electron microscopy has been used for many years, newer techniques such as DNA-metabarcoding, near infrared spectroscopy, mass-spectrometry, or the application of artificial intelligence are developing rapidly. Some have already yielded reliable results and are being routinely implemented in certain honey laboratories, although a standardisation of methods across several laboratories or countries is still missing.

3.5.4.1. SEM

Electron microscopy can be performed to get high resolution images of pollen wall surface (SEM), or the cellular content of pollen grains (TEM). With SEM, the morphological characteristics of pollen grains and especially the ornamentation of the pollen wall is examined. This may assist with the identification of pollen types with similar morphologies, and help to analyse the evolutionary relationship within taxonomic groups (Dustmann and Von der Ohe 1993; Halbritter et al. 2018). In melissopalynology, SEM cannot replace traditional LM methods and/or determine relative abundances, but it can be a valuable tool for the identification of unknown pollen types in various honey samples.

During this research, multiple honey samples were examined in SEM. They were selected according to the relevance of unidentified pollen types after LM analysis. Additionally, the eight pooled honey samples from the publication “Comparing the acetolysed and hydrated methods for the pollen analysis of honey” (Chapter 4.B.) were examined with SEM to produce micrographs of the general pollen diversity. All honey samples were prepared with the acetolysis method (Halbritter et al. 2018), updated by (Koelzer et al. 2024) to completely dry out the material and get rid of organic substances such as sugar and pollenkitt. The acetolysed pollen in 96% ethanol was then pipetted onto an aluminium stub and sputter coated with gold in

an Argon atmosphere in order to create a conductive surface. In the SEM, a focused beam of high-energy electrons was emitted by a tungsten filament and directed onto the pollen sample, where it hit the surface and ejected secondary electrons. These secondary electrons together with some backscattered primary electrons, were absorbed by detectors and their energy was used to create a detailed image of the pollen wall surface. The electron microscope used during this research was a JEOL IT300. A graphic description of the preparation process can be found in the Appendix (Figure A7+A9).

3.5.4.2. DNA

DNA metabarcoding is a precise and efficient method to identify pollen diversity in honey (Bell et al. 2016). It can improve melissopalynological analyses, especially when pollen types are difficult to identify microscopically due to similar morphology or limited optical resolution.

First, DNA is extracted from honey in a multi-step process that eliminates sugar and other inhibiting compounds. After that, primers are applied to amplify target DNA regions with polymerase chain reaction (PCR). Five primer systems have been successfully used for DNA metabarcoding of pollen from honey before. They are the nuclear ribosomal regions ITS2 and ITS1, as well as the chloroplastic *trnL*, *rbcL* and *matK* regions (Richardson et al. 2015). They can vary considerably in base pair lengths. By using highly universal markers, a large variety of taxonomic groups can be identified. On the other hand, inter-specific discrimination may be compromised (Bell et al. 2016). The targeted DNA is sequenced using high-throughput technologies such as MiSeq Illumina technology (Fox et al. 2022; Noël et al. 2023). Sequences are then checked against available taxonomic references in the NCBI nucleotide database using BLAST technology. For the processing of sequence reads, quantitative thresholds are applied and operational taxonomic units (OTU) may be assigned (Bell et al. 2016).

As of now, there has not been a successful attempt to standardise DNA metabarcoding techniques across laboratories and countries, which limits its use for official analyses. It is however widely used in the academic research of honeybee nutrition, foraging behaviour, and as biomonitoring tool to assess the surrounding

plant diversity (De Vere et al. 2017; Bänsch et al. 2020; Fox et al. 2022; Noël et al. 2023).

3.5.4.3. NIRS

Smell and taste of honey derives from the distinct composition of polyphenols, carotenoids, flavonoids, and minerals in the nectar of the respective plant species (Escuredo et al. 2021). Determining these compounds with the help of near-infrared spectroscopy (NIRS) may point to the principal botanical source of the sample. While mainly suggested for the detection of adulterations with rice and maize syrup and beet root sugar (Soares et al. 2017), NIRS may also help with the identification of unifloral honey. For this, near-infrared light with wavelengths ranging from 700 to 2500 nm (Escuredo et al. 2015; Bodor et al. 2021) is directed at a sample on a cam-lock cup and the reflectance of compounds is measured. Combined with multivariate statistical analyses, it is a rapid, non-destructive, low-cost analytical technique for a multitude of food products (Escuredo et al. 2021). Good results have been obtained by (Escuredo et al. 2015) for the detection of minerals and some pollen types such as Erica, Eucalyptus, and Castanea. On the other hand, NIRS alone is not effective enough in differentiating honey samples for the analysis of botanical and geographical origin and a combined approach together with physico-chemical parameters and light microscopy is recommended (Bodor et al. 2021).

3.5.4.4. MALDI-MS

Matrix Assisted Laser Desorption/Ionization mass spectroscopy (MALDI-MS) has been used to determine the geographical origin of honey through the analysis of protein mass spectra. The protein “fingerprint” of a sample is created and compared to the spectral barcodes of a known reference collection (Wang et al. 2009). It was also used to determine the botanical origin of corbicular pollen pellets (Braglia et al. 2024). However, the chemical marker profile may be altered by environmental influences and certain beekeeping practises, allowing to question the reliability of spectroscopic results (Madesis et al. 2014).

3.6. AIMS OF THIS RESEARCH

Urban honey presents particular challenges in tracing its botanical and geographical origin, especially so in a globalised world with changing climate. This research aims at developing a fundamental understanding of what defines urban honey from a melissopalynological perspective. The results of detailed light microscopic pollen analyses will shed light on the diversity of foraging resources used by honeybees in Central European cities today, with a focus on Vienna.

To account for the heterogeneity of urban environments, the analysed samples are from three consecutive years (2020–2022) and different harvesting times between beginning of June and end of August. For the full list of samples, see Appendix Chapter I and Figures A1–A5. The twelve selected cities vary in population size from approximately 30,000 to 2 million inhabitants. In larger cities, honey from multiple apiaries allows for a comparison between densely built inner-city and suburban areas. Mass foraging resources for city-kept honeybees are identified. Besides, the frequency of ornamental and neophytic plants in urban honey is analysed, in order to evaluate their potential as “marker pollen” for urban origin.

A second pillar of this study is a comparison of the advantages of different melissopalynological preparation methods (hydrated and acetolysed). The aim is to evaluate their suitability in the context of identifying a very diverse set of pollen types. Lastly, this research describes how open access reference databases support pollen identification, particularly in light of the increasing presence of non-native ornamental plant species.

Ultimately, this research shall serve as a basis for the assessment of the botanical and geographical origin of urban honey in future routine analysis.

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4. PUBLICATIONS

A. INTEGRATION OF THE POLLEN DATABASE PONET INTO *PALDAT* WITH NEW FEATURES FOR LIGHT MICROSCOPY

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Integration of the pollen database *PONET* into *PalDat* with new features for light microscopy

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Abstract
Free access to scientific data, knowledge and technology transfer has become more and more important in science, at universities, governmental institutes, and in our society in general. In an interdisciplinary project initialised by palynologists at the University of Vienna and the Austrian Agency for Health and Food Safety Ltd (AGES), the world's most comprehensive database *PalDat* has been expanded by more than 2000 datasets from the *PONET* database, comprising high resolution light microscopy (LM) micrographs of hydrated pollen. The main aim of this fusion of knowledge was to share digital data in a secure way and to ensure users of various disciplines free access to reliable pollen data. The project was finally completed in 2021. In the course of the expansion, additional data entry fields regarding LM description have been added, and new features, such as a search function for orders and families, have been implemented in the new version 3.4 of *PalDat*. A global online submission and publication tool for palynological data to *PalDat*, including review and editorial process, assures easy knowledge transfer and facilitates a continually increasing number of datasets and taxa contributed from scientists all over the world. The free accessible online pollen database *PalDat* is maintained by members of the Division of Structural and Functional Botany (University of Vienna).

Keywords: *melissopalynology, pollen analysis, electron microscopy, terminology, identification, dataset, submission*

The identification of pollen grains is crucial in many branches of applied science, e.g. in melissopalynology (Armbruster & Jacobs 1934; Sawyer 1981), aerobiology (Bastl et al. 2017), and forensics (Mildenhall et al. 2006; Weber & Ulrich 2016). As researchers of these disciplines, we rely heavily on broad access to reference pollen data in literature and databases, to identify the botanical origins of various pollen types in a sample.

There are several online pollen databases for this reason, most of which focus on certain geographic regions, i.e. Australasian Pollen and Spore Atlas (APSA Members 2007), disciplines, i.e. aerobiology (Bucher & Kofler 2023), or specific preparation methods, i.e. Pollen-Wiki (Stebler 2023).

As individual pollen databases are usually run by different institutes, some may exist parallel to each other in the same region, as it was the case with the two most important Austrian online databases *PONET* and *PalDat* (2000 onwards; https://www. paldat.org/). In 1992, melissopalynologists at the Institute of Apiculture and Bee protection at the Aus- trian Agency for Health and Food Safety Ltd (AGES) started to build up a pollen reference collection as backbone for applied honey analysis. The data and

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images gathered since then were combined into a freely accessible online database called *PONET* (Moosbeckhofer et al. 2013). *PONET* comprised datasets with light microscopic images of more than 2000 plant species, primarily of Central European distribution and relevant to honey pollen analysis.

Around the same time, another pollen database was established in Austria – *PalDat*. Started by a group of palynologists at the Department of Botany and Biodiversity Research at the University of Vienna in 1997, *PalDat*'s aim has been to provide excellent data and images to scientists and prac- titioners dealing with pollen and spores (Weber & Ulrich 2017). This is done by combining pollen mor- phological and structural characteristics from light microscopy (LM, hydrated and acetolysed pollen) and electron microscopy (scanning electron microscopy [SEM] as well as transmission electron microscopy [TEM]). The focus of *PalDat* has so far been on SEM images.

Unfortunately, in 2019, cost-intensive new information technology (IT) requirements made the further operation of *PONET* impossible and the website was shut down. With the attempt to save the extensive pollen data for scientific and applied use, palynologists from AGES and University of Vienna formed a unique cooperation between a governmental agency and a university institution, to integrate the data from *PONET* to *PalDat*. The database merge was finally completed in early 2021, after each individual dataset had been peer-reviewed through the submission process in *PalDat*. The integration and publication of these datasets now allows a large number of international users to utilise them for identification purposes.

For pollen identification, the 'Combined Search' tool is routinely used by palynologists. In this advanced search tool, specific pollen characteristics (i.e. size, ornamentation, type of apertures) can be selected and the results are displayed in a taxonomic tree (including total numbers of investigated species with these combined characteristics).

As of 2022, *PalDat* is the most comprehensive database available, offering reference data on a wide range of taxa using multiple methods and covering a worldwide distribution. The expansion in LM images and users in applied disciplines made a new set of parameters on *PalDat* necessary. The new version – *PalDat* 3.4 – now includes new fields for size in LM (hydrated) and dominant orientation, in the submission, publication and search tool. Com- pared to *PONET* and other LM pollen databases, for example, Pollen-Wiki (Stebler 2023), the com- bined search options on *PalDat* provide a complete range of description fields that aid in light microscopic pollen identification.

Pollen terminology used for light microscopic analysis

Identification of pollen taxa is based on the characteristics of the pollen grains: size, number and position of apertures, and structure and ornamentation of the pollen wall. Their characteristics are highly consistent within a plant species but may vary widely between different plant genera and families (e.g. Sarawichit 2012; Ulrich et al. 2012; Hesse & Blackmore 2013; Halbritter et al. 2018). The characteristics of an individual pollen grain should be described by using a standardised terminology, based on the book *Illustrated Pollen Terminology* (Halbritter et al. 2018). The book (Figure 1) is open access and available as online pdf on the SpringerLink website (https://link.springer.com/content/pdf/10.1007/978-3-319-71365-6.pdf?pdf=button), and provides detailed descriptions and illustrations of pollen terms for LM, SEM, and TEM. In addition, a shortened version is available on the *PalDat* website on the 'Terminology' page under the link 'Tools for Pollen Description' (https://www.paldat.org/static/illustrated_pollen_terminology_2020.pdf).

The newly specified parameters for LM in *PalDat* 3.4 are discussed in the following section.

'*Pollen Unit*' – '*Size (Pollen Unit)*' *and* '*Size of Hydrated Pollen*'

The five existing categories under 'Size (Pollen Unit)', ranging from 'very small' to 'very large', are primarily measured on pollen in hydrated state investigated with SEM, or acetolysed pollen samples analysed with LM and SEM. With the variability of size due to the various preparation methods, a new field for 'Size of Hydrated Pollen' in LM was needed for the integration of datasets from the former database *PONET*. Therefore, several new size categories for hydrated pollen in LM were included in *PalDat* 3.4. The new size categories for hydrated pollen in LM range from '< 11 µm' to '40 µm' in steps of 5 µm, followed by '41–50 µm', '51–100 µm' and '> 100 µm'. On the 'Combined Search' page (Figure 2), these size categories can be selected in a dropdown list in the field 'Size of Hydrated Pollen'.

How to measure pollen grains during LM pollen analysis

The size of an unidentified pollen grain can be helpful to narrow down search results. Pollen grains

Figure 1. *Illustrated Pollen Terminology* (Halbritter et al. 2018). Screenshots showing the cover page and one page from the chapter 'Ornamentation'.

can be measured using LM with either a calibrated imaging software on the computer or by using a micrometre scale slide. All measurements are performed in optical cross-section. Orientation and *P/E* ratio of pollen grains should be considered when measuring pollen size. The length of the polar axis and the equatorial diameter (Figure 3) are the most important measurements in prolate and oblate pollen grains. Isodiametric, spheroidal pollen grains, where polar axis and equatorial diameter are of equal length, are measured in at least one axis for identification purposes (Figure 4). For measuring other pollen types (e.g. ulcerate, sulcate, tetrads) respective literature is available (e.g. Sivak 1973; Goldblatt et al. 1991; Grímsson & Zetter 2011; Halbritter et al. 2018).

To publish data in *PalDat* the size of the longest and shortest length of both polar axis and equatorial diameter is required in the submission process (Figure 5). For the description of a pollen type, at least five individual pollen grains should be measured in polar and equatorial view each. If pollen grains of the respective plant species fit in two or more size categories (i.e. due to size variability within the anther), the species will then show up in the search results in any of the determined size categories. In addition to the existing entry field 'Size of Hydrated Pollen' (longest axis of hydrated pollen), four new fields concerning pollen size have been added, namely for the 'shortest/longest polar axis in equator- ial view (LM)', and for the 'shortest/longest diameter in equatorial or polar view (LM)'. For further information see *PalDat*'s 'Instructions for Authors', which can be downloaded as pdf from the *PalDat* website (https://www.paldat.org/static/paldat_instructions_for_authors.pdf).

Polarity and P/E *ratio – understanding the polarity and shape of hydrated pollen*

The pollen polarity is determined by the orientation of the immature pollen grains (microspores) in the tetrad stage (Figure 6). The polar axis of a microspore/pollen grain runs from the proximal pole, orientated towards the tetrad centre, to the distal pole of the microspore/pollen grain. The pollen

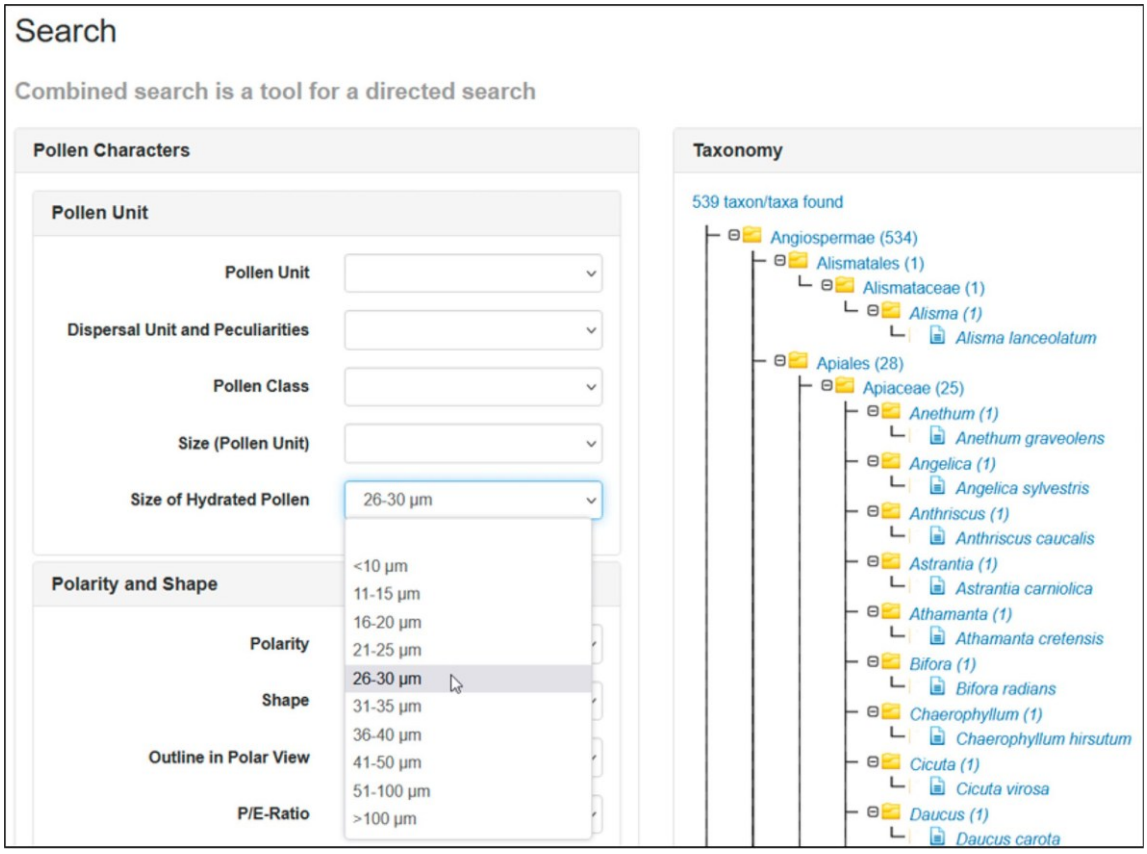

Figure 2. Screenshot 'Combined Search' with selected 'Size of Hydrated Pollen'.

grains are divided by the equatorial plane (located at the microspore's centre) into a proximal (towards the tetrad centre) and a distal half. The polarity determines the polar and to the equatorial view of a

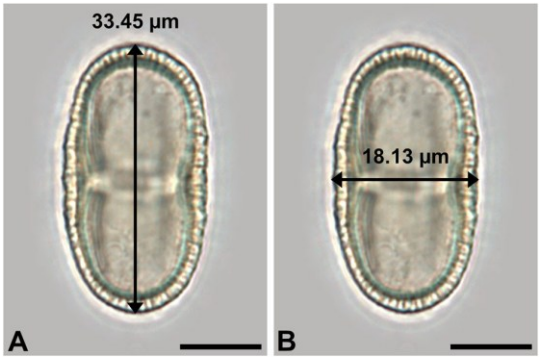

Figure 3. Measuring hydrated Apiaceae pollen (LM) in equatorial view: Length of polar axis (A) and length of equatorial diameter (B). Scale bars – 10 µm.

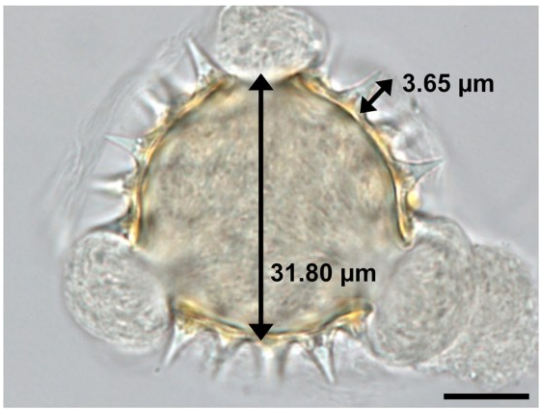

Figure 4. Measuring hydrated Asteraceae pollen (LM) in polar view. The size of ornamentation elements, such as echini, should be measured separately. Scale bar – 10 µm.

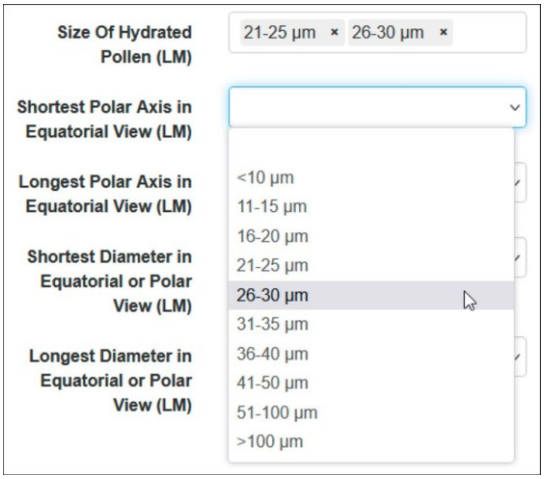

Figure 5. Screenshot of the diagnosis section in the submission tool: For 'Size of Hydrated Pollen (LM)', multiple categories can be selected.

pollen grain. Pollen with identical proximal and distal poles are termed isopolar pollen, whereas the proximal and distal poles are different in heteropolar pollen. Also, the shape and aperture position are directly related to the polarity of a pollen grain (Halbritter et al. 2018).

The pollen shape refers to the three-dimensional form of a pollen grain in relation to the *P/E* ratio. The *P/E* ratio refers to the length of the polar axis between the two poles compared to the equatorial diameter

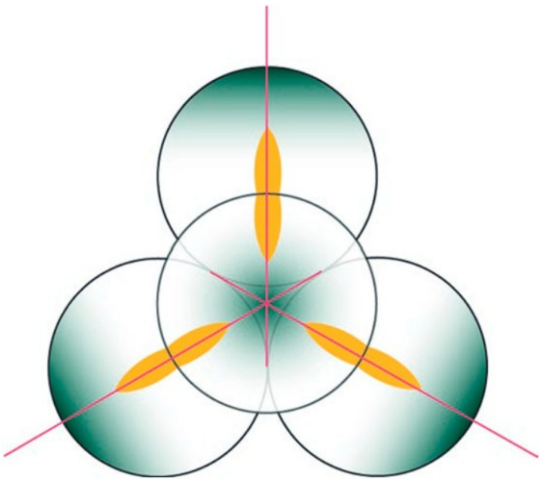

Figure 6. Illustration showing the orientation of microspores within the tetrad during pollen development (Halbritter et al. 2018).

and are measured in hydrated pollen grains. According to Halbritter et al. (2018), the *P/E* ratio can be described as isodiametric (polar axis is ± equal to the equatorial diameter), prolate (polar axis is longer than the equatorial diameter), or oblate (polar axis is shorter than the equatorial diameter the *P/E* ratio).

Dominant orientation – polar, equatorial, and oblique view

For some branches of applied palynology, for example, melissopalynology, it can be useful to know the dominant orientation of hydrated pollen of a species within the sample. The orientation is determined by the *P/E* ratio of the pollen grain. Oblate pollen is mostly found to be present in polar view, whilst prolate pollen is mainly found in equatorial view (Figure 7). Spheroidal pollen grains with isodiametric *P/E* ratio may be oriented in either a polar or equatorial view, but they are usually also found without a specific orien- tation (oblique view, Figure 7C). In pantoporate pollen types (for example, Amaranthaceae species) polarity and *P/E* ratio are undefined and therefore have no dominant orientation.

'*Ornamentation in LM*' *– a more detailed description of the pollen wall ornamentation*

As the resolution of light microscopes is constantly increasing due to advanced technology, descriptive terms for the ornamentation of the pollen walls also become more diverse. While the former pollen database *PONET* was limited to only seven ornamentation groups, it is now possible to choose and search in *PalDat* under 'Ornamentation LM' for 26 individual ornamentation types (Figure 8). All ornamentation types should ideally be observed and described using a ×100 oil objective lens. For an accurate description of ornamentation elements during LM analysis, the book *Illustrated Pollen Terminology* (Halbritter et al. 2018), or the online pdf 'Pollen Terminology' provided on the *PalDat* website, are useful tools for palynologists. A short description and illustrations for each term are the prerequisites for consistent, unambiguous descriptions of pollen grains.

Taxonomic search

A taxonomic search tool (Figure 9) has been added in the latest version of *PalDat* 3.4 to the section 'Search Data'. In addition to the 'Alphabetical Search' of plant genera and the 'Combined Search' (Figure 2), it allows users to search for specific plant orders (for example, Brassicales, Lamiales) or plant families

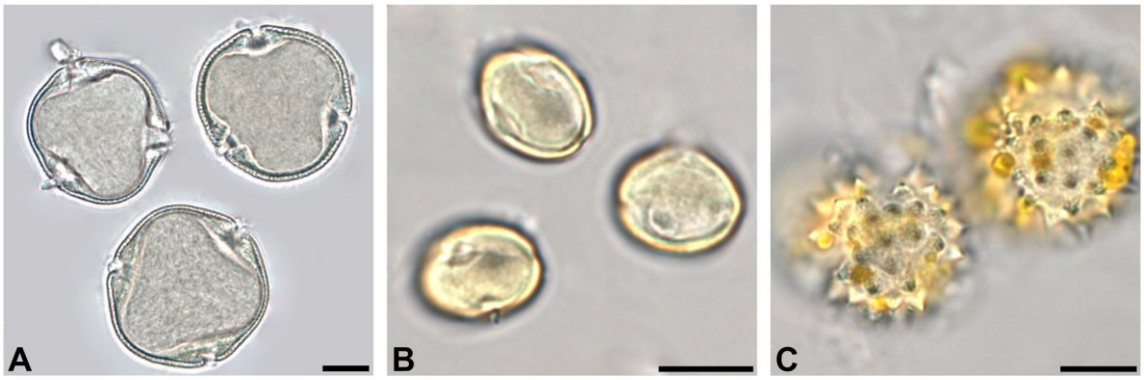

Figure 7. Dominant orientation of selected pollen types in a melissopalynological sample: *Tilia* sp. in polar orientation (A), *Castanea sativa* in equatorial orientation (B), and Asteraceae in oblique orientation (C). Scale bars – 10 µm.

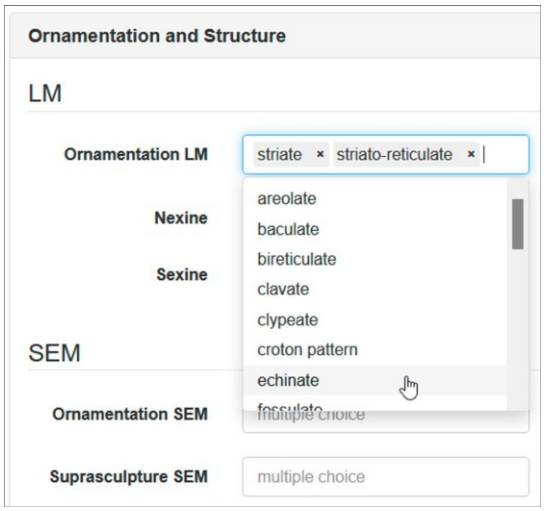

Figure 8. Screenshot of the diagnosis section in the submission tool: Multiple ornamentation types can be selected under 'Ornamentation LM'.

(for example, Brassicaceae, Oleaceae), and to compare pollen of closely related species/genera. The results are displayed in a taxonomic tree (includ- ing numbers of investigated species), which helps to understand pollen characteristics in the context of plant phylogeny. This tool has an enormous potential and may be used, for example, in testing how lineage specific certain pollen types are, or in testing phyloge- netic signals in certain pollen types.

Conclusions

The integration of *PONET* into *PalDat* resulted in a remarkable increase of plant species in the pollen

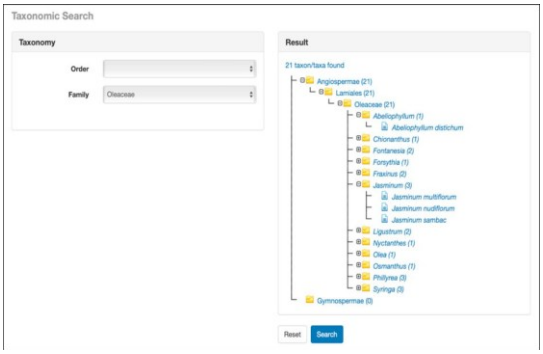

Figure 9. Screenshot showing the 'Taxonomic Search' tool with taxonomic tree of results.

database. At present (31 March 2023) the database provides 35 817 pictures of 4491 species belonging to 1789 genera, and the number of datasets continu- ally increases.

Registered users can easily publish their data online on the *PalDat* website and share them with a larger scientific community through a free global online submission and publication tool for a wide spectrum of pollen data. Authors can either submit a new dataset or contribute as co-author(s) to an existing dataset, i.e. by adding LM pictures. New submissions, as well as all changes made in an exist- ing publication undergo a review process and edi- torial check, before being published. Each dataset ideally includes a detailed pollen description, light and electron micrographs, images of the plant/inflor- escence/flower, and relevant literature. Online publi- cation on the *PalDat* website might be especially useful if the data cannot be used for publication in

a peer reviewed journal but may still contribute to the open exchange of knowledge and data.

With the fusion of the two pollen databases, *PalDat* has strengthened its role as an eligible online publication platform for LM images for users in various disciplines.

Acknowledgements

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Disclosure statement

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B. COMPARING THE ACETOLYSED AND HYDRATED METHODS FOR THE POLLEN ANALYSIS OF HONEY

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Comparing the acetolysed and hydrated methods for the pollen analysis of honey

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Abstract
Melissopalynology is an integral part of honey analysis and indicates the honey's botanical and geographical origin. Two preparation methods for light microscopy are commonly and interchangeably used in laboratories: acetolysis and hydration of honey samples. In this study, an extended comparison of the two methods was performed. Eight honey samples from eight locations in central Europe were analysed according to both standard procedures. We identified the pollen grains with 1000× magnification to the lowest possible taxonomic rank and counted out the relative abundances of each pollen type in the sample. In addition, potential other biases that might affect the results were investigated by analysing one honey sample multiple times with each method, consecutively. Across all honey samples, more pollen types were identified with the hydrated method (136) compared to the acetolysed method (95). For the hydrated method, less washing steps were necessary and a higher amount of pollen grains remained. No taxa-specific loss could be observed, apart from *Myosotis* sp., which was present in significantly lesser amounts in acetolysed compared to hydrated samples. This study shows a detailed analysis of the advantages and disadvantages of the two methods, which are also highlighted in a side-by-side comparison box. Both methods are equally suitable to perform melissopalynological analysis of the most common pollen types and pollen type combinations in honey. However, to identify the largest variety of pollen types in honey samples, the hydrated method is more efficient.

Keywords: *abundances, acetolysis, floral resources, foraging plants, honeybees, light microscopy, melissopalynology, methodology, palynology*

Pollen analyses have been an essential part of environmental research for many years in the fields of aerobiology (Oteros et al. 2013; Galán et al. 2014; Bastl et al. 2017), palaeobiology (Faegri & Iversen 1975; Grímsson et al. 2020; Geier et al. 2023), climatology (Straka 1975; Chevalier et al. 2020), and forensic palynology (Mildenhall et al. 2006; Weber & Ulrich 2016). In melissopalynology, they are used in food safety laboratories as important routine tool for the analysis of honey and other honeybee products (Maurizio 1975; Vorwohl 1995; von der Ohe et al. 2004).

In fact, one of the earliest pollen analyses of recent material has been conducted by apiarists in an attempt to identify foraging plants of honeybees. Identification literature for beekeepers with descriptions and drawings of unstained hydrated pollen grains date back to the 1930s, e.g. Armbruster and Jacobs (1934–1935), coinciding with the increasing availability of microscopes with sufficient magnification. The method of hydrating honey samples in

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glycerine or glycerol gelatine is simple yet effective and has been used by apiarists and melissopalynologists for more than a century to determine the botanical and geographical origin of honey, e.g. by Louveaux et al. (1970, 1978), Sawyer (1988), Vorwohl (1995), the DIN (Deutsches Institut für Normung) (2002), and von der Ohe et al. (2004).

Since the mid-twentieth century, acetolysis of pollen samples is a much-utilised light microscopy (LM) method, besides simply hydrating a sample in water or glycerine. Erdtman (1943) stated that there are several methods of pollen analysis available, but acetolysis would deliver the most beautiful results. Acetolysis, as described in detail by Erdtman (1960) and revisited by Hesse and Waha (1989), can be applied to any pollen sample and is the method of choice for palaeobotanists, climatolo- gists and archaeologists due to the age of their ana- lysed samples and the organic debris such samples commonly contain (e.g. Straka 1975).

Today, using the hydrated method is standard in melissopalynological investigations (von der Ohe et al. 2004). However, which method is actually used for honey pollen analysis depends largely on the training of the analyst, the common practise in the specific laboratory, the geographic region, and the available literature.

Differences in the results between the two methods might occur qualitatively during the identification of pollen types, when they highlight certain characteristics of a pollen grain (e.g. pollen wall sculpture), or quantitatively, when a statistically relevant amount of pollen grains is counted out to track the relative abundance of each pollen type in the sample, e.g. DIN (Deutsches Institut für Normung) (2002) and von der Ohe et al. (2004). These differ- ences might impact the integrity of results in applied melissopalynology, where the occurrence of certain pollen types points to the geographical origin of the honey, and/or where absolute numbers and/or relative percentages are used to classify the botanical origin. Some aspects of sample quality and pollen identification level in regard to the two methods were described before (Lieux 1980), yet previous publications on the comparison of the two methods are scarce and often restricted to single honey samples or certain geographical locations (Gadbin 1979). More often, after pollen analysis was performed with a hydrated sample, a subsample was additionally acetolysed to confirm the botanical origin (e.g. Durán-Escalante et al. 2023).

The aim in this study was to elaborate on the similarities, differences, and potentials of both methods by conducting melissopalynological analyses from multiple samples and multiple places, both in acetolysed and hydrated state. To this end, honey from four countries was investigated. In addition, one selected sample was repeatedly prepared and ana- lysed five times consecutively with both methods to investigate possible influences on the results other than the preparation method.

Material and methods

Study design

In the first part, pollen analyses of the same eight honey samples in hydrated state as well as in acetolysed state were performed. In the second part, one selected honey sample was independently prepared and counted in four more repeats, in order to check for possible variance within one preparation method (see Table I).

Material

Eight honey samples from eight different cities in central Europe were analysed. The cities were Munich (Germany), Bratislava (Slovakia), Ljubljana (Slovenia), as well as Vienna, Linz, Salzburg, Innsbruck, and Klagenfurt (all located in Austria). All extracted honey samples were harvested in the summer of 2020 by local apiarists. For the purpose of depicting a greater variety of pollen types in the sample of each city, honey from several apiaries within the respective city was mixed. The number of apiaries that were represented in the final eight honey samples ranged from two (Ljubljana, Linz, Salzburg, Innsbruck) to 18 (Vienna) according to availability. All samples were thoroughly homogenised before further handling.

For the second part of the study, the honey sample from Munich was analysed four more times. The sample from Munich was selected as it showed the largest variety of pollen types among all analysed samples, with morphologies ranging from very small to very large size.

Acetolysis method (LM)

Acetolysis of samples was performed after Halbritter et al. (2018), with some adaptations for melissopalynology. For this study, 10 g of honey were added to approximately 30 ml of warm water in a small beaker and thoroughly dissolved on a heated magnetic stirrer for 3 min. The sample was transferred into a 12 ml round-bottom centrifuge glass tube and centrifuged at 3000 rpm (1107 × *g*) for 3 min. The supernatant was decanted and the rest of the solution was added, after

Table I. Study design

	Cities								Repetitions				
	Bratislava	Innsbruck	Klagenfurt	Linz	Ljubljana	Munich	Salzburg	Vienna	Munich 1	Munich 2	Munich 3	Munich 4	Munich 5
Acetolysed	1M_Brat_acet	1M_Inns_acet	1M_Klag_acet	1M_Linz_acet	1M_Ljub_acet	1M_Muen_acet*	1M_Salz_acet	1M_Wien_acet	1M_Muen_acet*	2M_Muen_acet	3M_Muen_acet	4M_Muen_acet	5M_Muen_acet
Hydrated	1M_Brat_hyd	1M_Inns_hyd	1M_Klag_hyd	1M_Linz_hyd	1M_Ljub_hyd	1M_Muen_hyd*	1M_Salz_hyd	1M_Vien_hyd	1M_Muen_hyd*	2M_Muen_hyd	3M_Muen_hyd	4M_Muen_hyd	5M_Muen_hyd

*Honey sample from Munich was considered the first repetition and is therefore identical.

which it was centrifuged and decanted again until completed. Before acetolysis, the concentrated pollen material was washed with acetic acid to remove the water and centrifuged at 4000 rpm (1968 × *g*) for 3 min. After the acetic acid was carefully decanted, acetolysis mixture consisting of nine parts acetic anhydride and one part concentrated sulphuric acid was added to approximately two thirds of the height of the glass tube. The sample was then placed in an ultrasonic water bath at 80 °C for 10 min. Afterwards, the tube with the acetolysed sample was taken out and centrifuged for 6 min at 3000 rpm (1107 × *g*). The supernatant acetolysis mixture was decanted, a drop of acetic acid was added and vortexed before washing the sample once more in acetic acid. After 3 min at 4000 rpm (1968 × *g*), the acetic acid was decanted and a final washing step with deionised water was performed. After decanting the tube for a last time, it was placed upside down on a filter paper for at least 10 min. Finally, 100 µl of deionised water was added to the pollen sample and pipetted onto a heated object slide. When the water was nearly completely evaporated, a drop of Kaiser's glycerol gelatine (Mulisch & Welsch 2015) was placed on the sample and it was covered with a 20 mm × 20 mm cover glass.

Hydration method (LM)

An adapted version of the standardised method of melissopalynology according to von der Ohe et al. (2004) was used to perform a detailed LM pollen analysis. From each homogenised honey sample, 10 g were mixed with 30 ml of deionised water and placed on a magnetic stirrer for 5 min. The solution was centrifuged at 3000 rpm (1107 × *g*) for 3 min in a 12 ml pointed centrifuge glass tube and decanted. These steps were repeated until the solution was completed. The sugary deposit was carefully removed using a pipette. The remaining pollen pellet was resuspended in 100 µl of deionised water. As the amount of pollen was very high at this stage, only approximately half of the suspended pollen was pipetted onto an object slide. This reduced the risk of problematic overlay of pollen grains caused by too much material used. The object slide was then dried on a heating plate and in a final step, the sample was embedded in one drop of Kaiser's glycerol gelatine (Mulisch & Welsch 2015), covered with a 20 mm × 20 mm cover glass and let rest for 24 h.

Pollen identification and counting

LM analysis was performed with an Olympus BX50 with attached digital camera. At least one grain of

each distinct pollen type was documented, which is recommended for a later identification attempt, or when proceeding with the analysis at another timer. For documentation, micrographs were taken at a 1000× magnification and in three different optical sections: top view and bottom view for details of the pollen wall sculpture and apertures, and the optical cross-section for pollen wall thickness and measuring the pollen diameter (Koelzer et al. 2023). If necessary for correct identification, we con- sulted online pollen databases PalDat (2000 onwards) and Pollen-Wiki (Stebler 2023), as well as relevant literature (e.g. Reille 1992; Beug 2004). Pollen grains were also counted out with 1000× mag- nification and up to a total amount of 500 in both methods. To guarantee homogeneous examination of the slide, counting was done in a matrix of multiple random equidistant lines, as in von der Ohe et al. (2004). Spores, algae and other palynomorphs were not considered. Grains of anemophilous Poaceae and Pinaceae were included into the 500 pollen grains for the purpose of a full comparison between the methods with all pollen types. Broken pollen grains were only included when they could be dis- tinctly assigned to an already identified pollen taxa.

All pollen identification and counting were performed by the same palynologically trained researcher, who has more than four years of experi- ence in analysing both acetolysed and hydrated honey samples.

Results

All eight honey samples were analysed using the acetolysed as well as the hydrated method. The identified taxa as well as their relative abundances in the honey samples are seen in Supplemental Table I (acetolysed) and Supplemental Table II (hydrated). Figures 1–4 show the 20 most abundant pollen types across all pollen countings.

Appearance and condition of pollen samples in LM

During the process of acetolysis, as expected, the cytoplast dissolved, and the pollen wall remained. It also dissolved organic debris in the sample, which left the background much cleaner and highlighted the pollen grains. The brownish staining after acetolysis increased the visibility of details in the pollen wall structure and sculpture. In some cases, the margins of the aperture could be much better observed in the acetolysed state, e.g. in Rosaceae species (Figure 5A, C). Another important feature of pollen grains is the aperture membrane, which can be smooth or ornamented. In some species, e.g. *Aesculus hippocastanum* (Figure 1Q–T), the ornamentation remained even after acetolysis, while in others, e.g. some Lamiaceae or monocots, this characteristic was in many cases lost.

In hydrated state, the pollen wall of some species was naturally coloured in a light yellow (e.g. *Salix* sp.; Figure 2K, L) or brownish (*Aesculus* sp.; Figure 1S, T) or even purple (*Cirsium*-type). In addition, the presence or absence of pollenkitt could only be observed in hydrated state and impacted colouring there. Pollenkitt of Asteraceae species, *Prunus* sp. (Figure 1K, L) or *Helianthemum* sp. was yellow, of *Koelreuteria* sp. brownish, and of *Amorpha fruticosa* orange-brown. Even within one plant family, different genera could be distinguished by natural colouring. In Rosaceae, for example, *Prunus* sp. (Figure 1K, L), appeared more yellow in colour than *Malus*-type (Figure 2O, P). Colour intensity in hydrated state also corresponded to the thickness of the wall. Pollen with very thin walls, e.g. *Myosotis* sp. (Figure 1O, P) or *Begonia* sp. (Figure 4S, T), were especially translucent.

In hydrated pollen samples, the cytoplast did not dissolve. It remained within the pollen walls and slightly expanded when embedded in glycerol gelatine. The way in which the hydrated cytoplast protrudes the apertures posed a valuable tool for identification. In Rosaceae, such as *Prunus* sp. or *Malus*-type, it presented somewhat constricted at the tip (Figure 5B, D). In some species, also the contents of the cytoplast were a useful character- istic. Starch globules within the cytoplasm are a prominent microscopic feature in *Rumex* sp. (Figure 6).

Comparative quantitative analyses of pollen spectra

Across all eight honey samples analysed for this study, a total of 147 pollen types were identified. A lot more pollen types were identified with the hydrated method (136 out of 147, see Supplemental Table II, compared to the acetolysed method (95 out of 147, see Supplemental Table I). The identified pollen types within all acetolysed and hydrated samples were of 69 individual plant families altogether: 68 out of 69 families with the hydrated method, compared to 47 out of 69 families with the acetolysed method. In the honey sample from Munich, a total of 52 plant families was ident- ified (see Supplemental Table III), with 48 out of 52 (hydrated method), compared to 40 out of 52 (acetolysed method). Across all samples, the families comprising the most identified taxa were Asteraceae

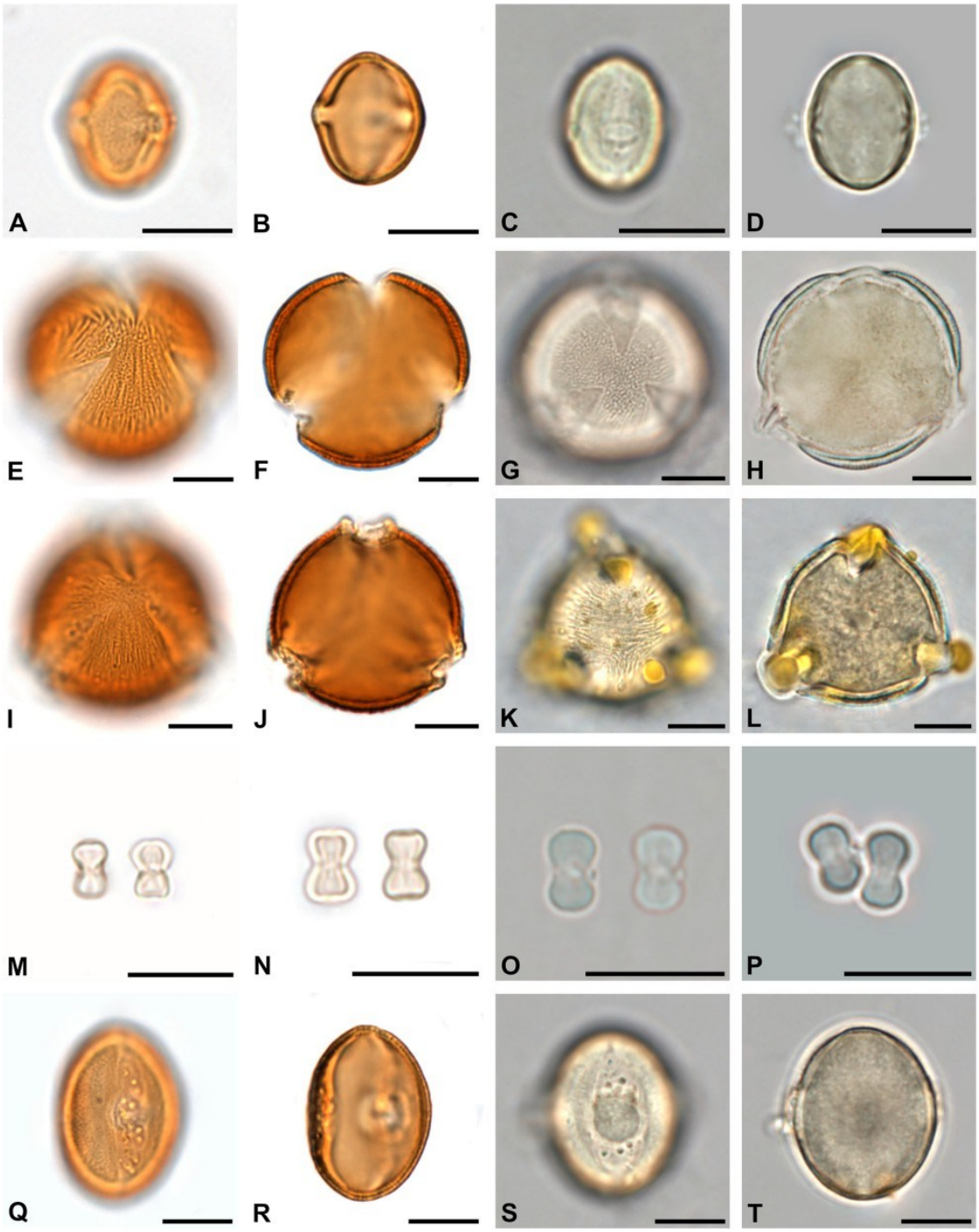

Figure 1. Some of the most abundant pollen forms in acetolysed (left) and hydrated (right) state. A–D. *Castanea sativa* in equatorial view. E–H. *Acer* sp. in polar view. I–L. *Prunus* sp. in polar view. M–P. *Myosotis* sp. in equatorial view. Q–T. *Aesculus hippocastanum* in equatorial view. Scale bars – 10 µm.

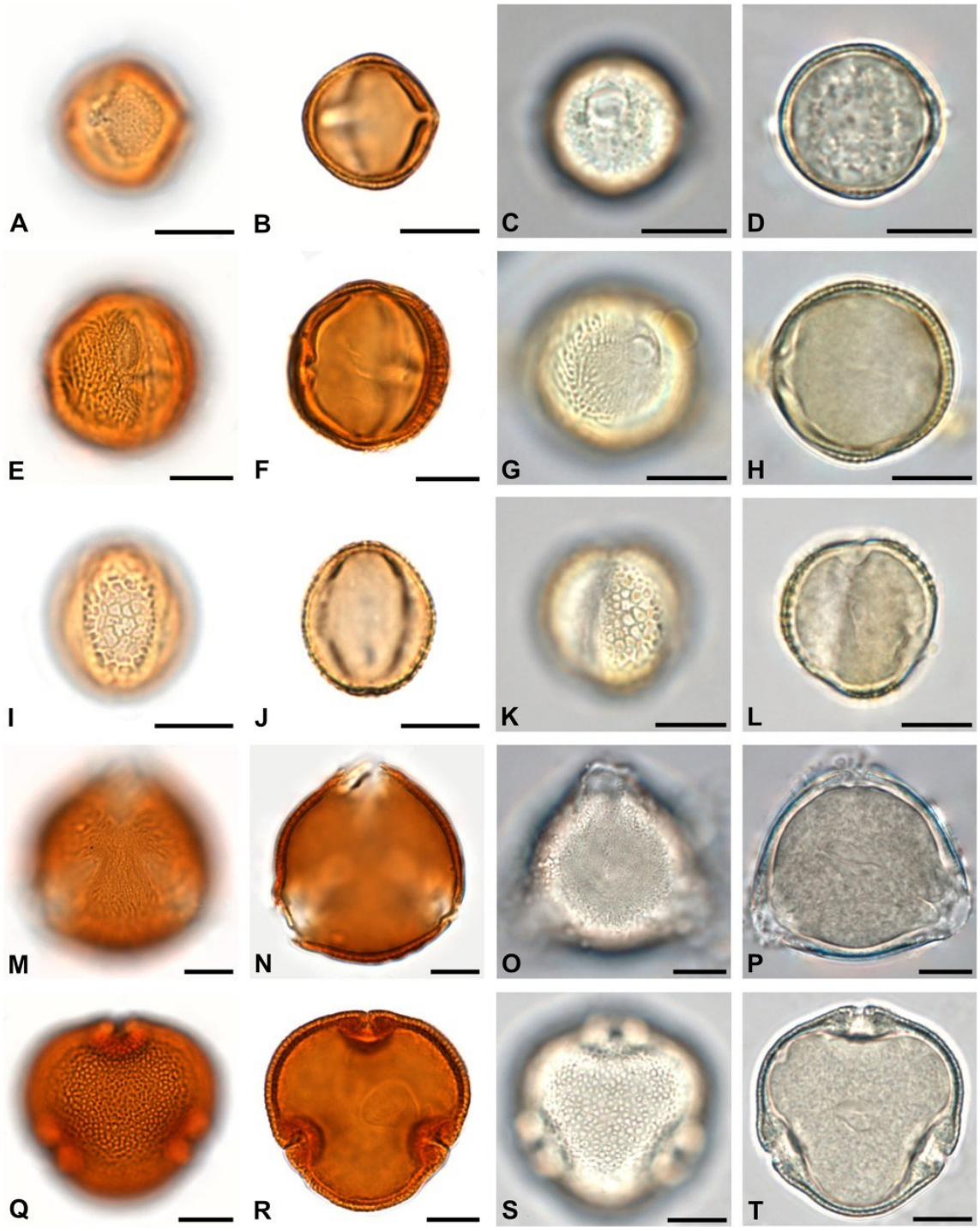

Figure 2. Some of the most abundant pollen forms in acetolysed and hydrated state. A–D. *Filipendula* sp. in equatorial view. E–H. *Ailanthus altissima* in equatorial view. I–L. *Salix* sp. in equatorial view. M–P. *Malus*-type in polar view. Q–T. *Tilia* sp. in polar view. Scale bars – 10 µm.

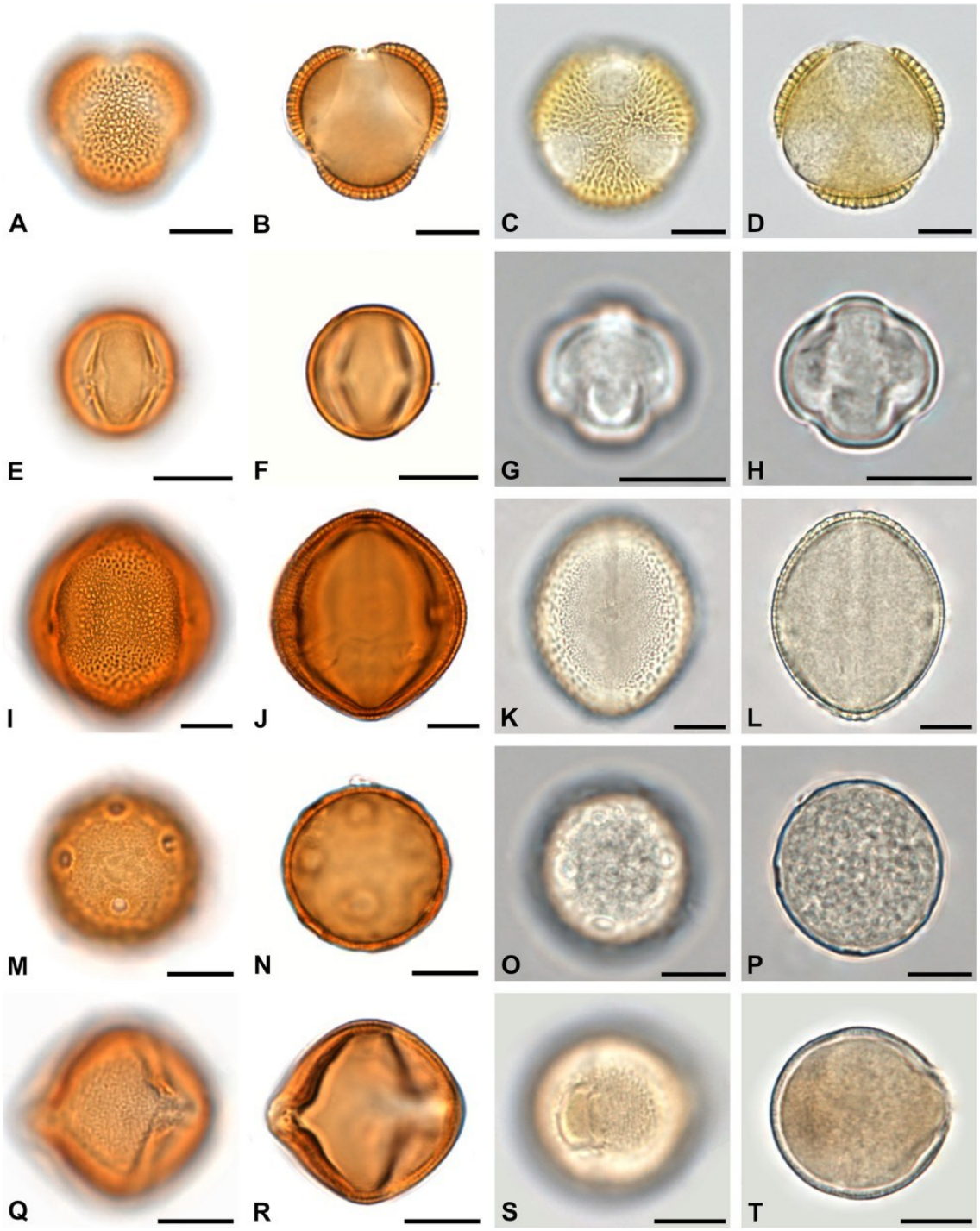

Figure 3. Some of the most abundant pollen forms in acetolysed and hydrated state. A–D. Brassicaceae in polar view. E–H. *Buddleja* sp. in equatorial view. I–L. *Parthenocissus* sp. in equatorial view. M–P. *Plantago* sp. in oblique view. Q–T. *Rubus* sp. in equatorial view. Scale bars – 10 μm.

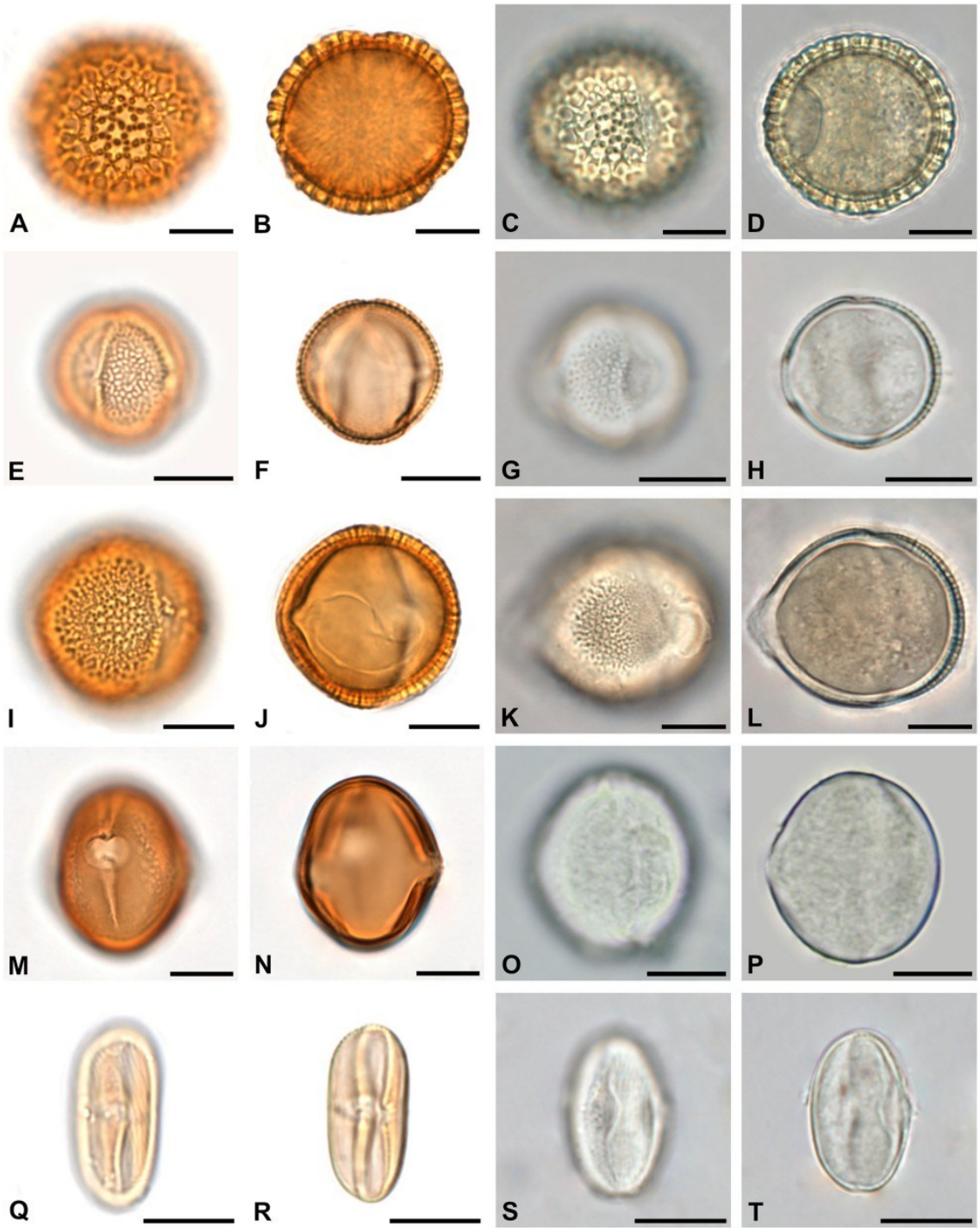

Figure 4. Some of the most abundant pollen forms in acetolysed and hydrated state. A–D. *Syringa* sp. in equatorial view. E–H. *Sambucus*- type in equatorial view. I–L. *Gleditsia* sp. in equatorial view. M–P. *Trifolium repens*-type in equatorial view. Q–T. *Begonia* sp. in equatorial view. Scale bars – 10 µm.

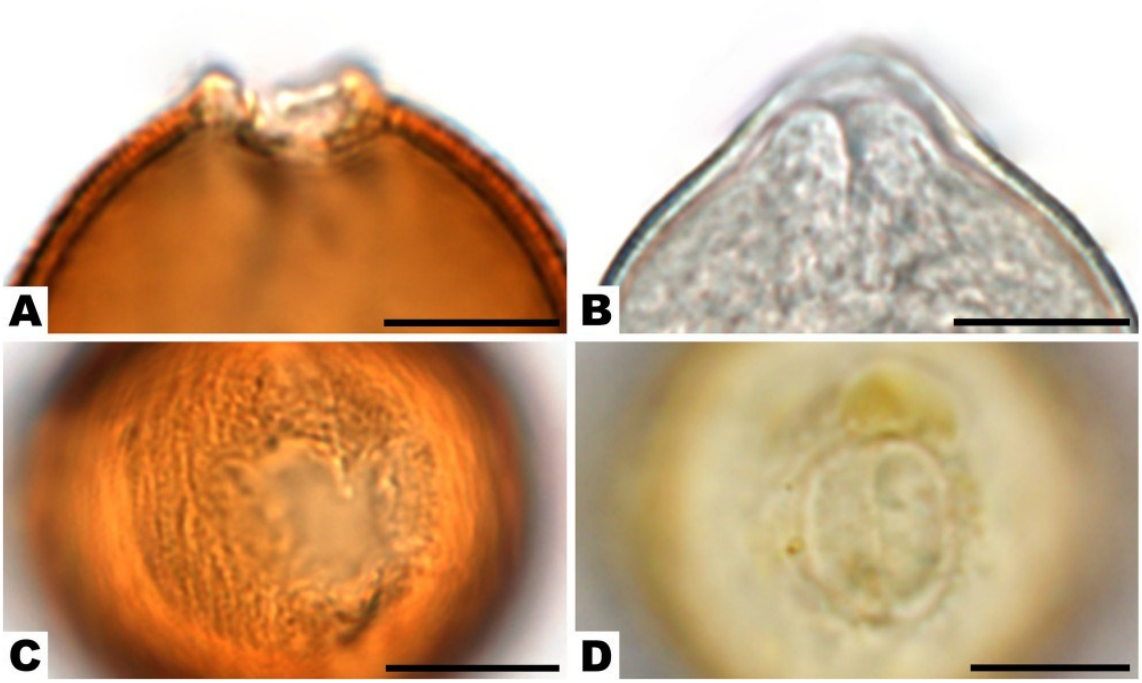

Figure 5. Apertures of Rosaceae species: A, C. *Prunus* sp. in acetolysed state. B. *Malus*-type in hydrated state. D. *Prunus* sp. in hydrated state. Scale bars – 10 µm.

(12), Fabaceae (10), Rosaceae (8), Boraginaceae (6), and Sapindaceae (5).

Within the honey from eight cities, the most abundant pollen type was *Castanea sativa* (657 pollen grains or 16.43% in total when acetolysed, compared to 553 or 13.83% when hydrated). Also, *Acer* sp. and *Prunus* sp. were among the most abundant, regardless of the method: *Prunus* sp. with 378 pollen grains (9.45%) when acetolysed and 237 (5.93%) when hydrated; *Acer* sp. with 356 pollen grains (8.90%) when acetolysed and 319 (7.98%) when hydrated. With a total of 263 pollen grains (6.58%) across all eight pollen counts, *Myosotis* sp. was the third most abundant pollen type in the hydrated samples, whereas only 19th in the acetolysed samples (39 grains or 0.98%).

In the second part of the study, the honey sample from Munich was prepared and analysed for five consecutive times on different days by the same person, in order to evaluate possible bias caused by the researcher. Also here, the plant families comprising the most pollen types were Asteraceae (7), Fabaceae (7), Rosaceae (7), and Boraginaceae (5). Notable differences between the repetitions of each method occurred in the Brassicaceae and Fabaceae families, as well as in some other pollen types. 'Brassicaceae' pollen ranged from 1.6–20% (acetolysed) and 5.6–6% (hydrated). *Brassica* sp. pollen ranged from 0–4.2% (acetolysed) and 5.2–6.2% (hydrated). Across the acetolysed repetitions, *Trifolium repens*- type could only be detected in one sample, while 'Fabaceae' pollen was present in all five samples. However, in the hydrated repetitions, *Trifolium repens*-type was present in all and 'Fabaceae' pollen in none. *Aesculus hippocastanum* ranged from 4.6–10.8% (acetolysed) and 4.2–7% (hydrated), *Castanea sativa* from 3.4–7.2% (acetolysed) and 3.4–4% (hydrated), and *Tilia* sp. from 4.4–13% (acetolysed) and 5.4–8.2% (hydrated).

Discussion

Across all honey samples analysed for this study, a remarkable difference in identified pollen types between the acetolysed and hydrated method could be observed. Around a third more pollen types were identified in the hydrated samples. This is due to the overall higher quantity of pollen which remained of the honey material after preparation and despite using only approximately half the volume of suspended pollen (equal to 5 g instead of 10 g) for the analysed object slide. Multiple steps of washing,

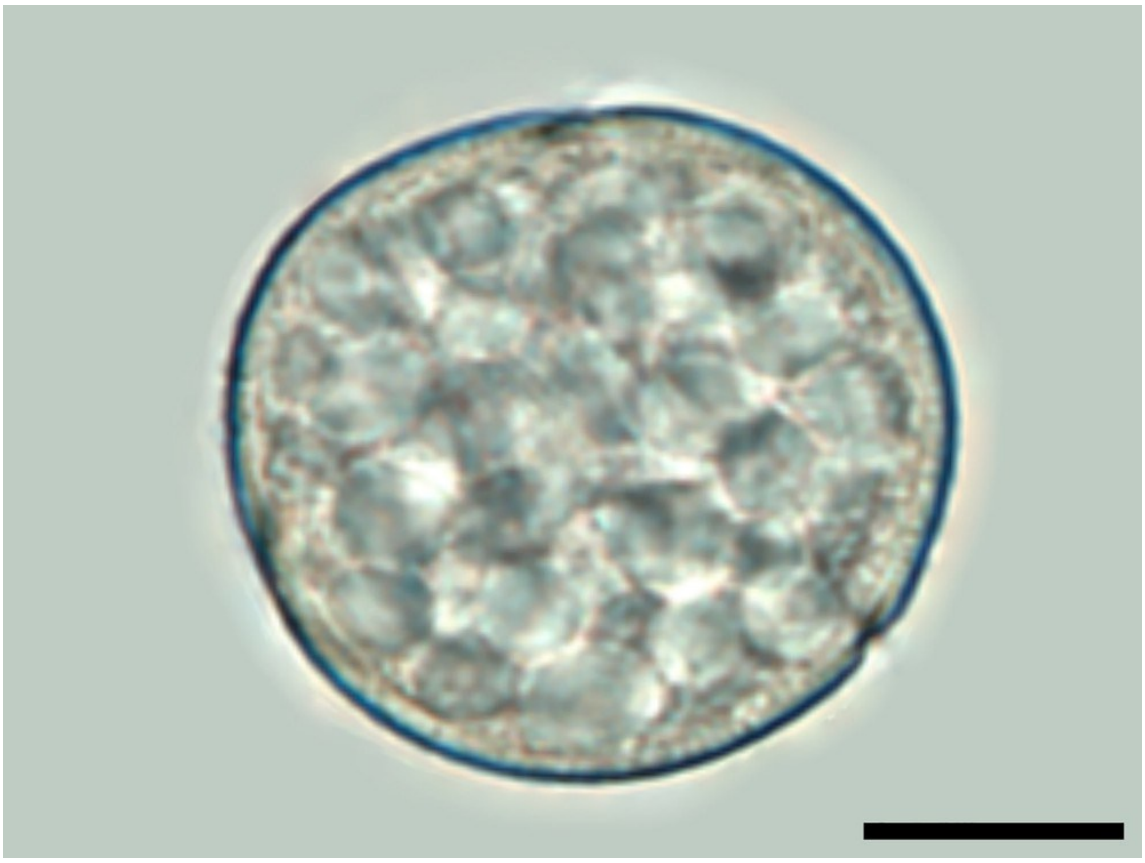

Figure 6. Numerous starch granules in the cytoplast of *Rumex* species. Scale bar – 10 µm.

centrifuging, and decanting of the supernatant in the process of acetolysis probably leads to pollen being gradually washed out, reducing the chances of encountering less frequent pollen types during analysis.

An extensive study by Sodl (2013), which focussed on pollen in fabrics, showed that no specific pollen taxa were lost during repeated washing steps with subsequent acetolysis. Similarly, in this study, it could not be observed that specific pollen types are lost to a larger extent in the acetolysed samples. Notable exception is *Myosotis* sp., where abundances varied drastically between the methods, despite careful microscopic screening. This was repeatedly observed with the several analyses of the honey sample from Munich. Pollen from *Myosotis* sp. is very small with less than 10 µm in size in addition to a very thin exine (Meudt 2016). Therefore, it may have been partially lost during acetolysis. It remains unclear whether this is because of the small size, or possible disintegration of the pollen grain due to the thin wall, or other factors. Other small pollen types with thin walls (e.g. *Begonia* sp., *Buddleja* sp., *Cynoglossum* sp., *Omphalodes* sp.) were present slightly more often in the hydrated method. In these taxa, differences cannot be quantified however, due to their little overall abundance in the samples. As noted by Ramalho and Kleinert-Giovan- nini (1986), smaller pollen grains might accompany the movement of the embedding material after adding the cover glass and counting in multiple random lines across the slide is important to avoid effects of differentiated distribution. With glycerol gelatine used for both preparation methods in this study and *Myosotis* sp. being encountered all over the slide in the hydrated samples, this is not likely to have caused the little abundance of *Myosotis* pollen in the acetolysed samples.

The depth of taxonomic resolution might account for some other variances in the list of identified pollen types. Generally speaking, certain pollen types may not be distinguishable on species level and a higher taxonomic name should therefore be used (e.g. 'Rosaceae' or '*Prunus*-type' instead of *Prunus domestica*) in order not to produce false positive results. Another way in which to describe potentially also paraphyletic groups is to assign a pollen type to an aggregate of similar looking pollen species (see Ramalho & Kleinert-Giovannini 1986), e.g. by calling it *Sambucus*-type instead of *Sambucus nigra* sensu stricto.

The identified taxonomic level of a pollen type might also vary with several repetitions. In this study, for example, some pollen grains were identified as *Trifolium repens*-type in one sample and Fabaceae in another sample. While *Trifolium repens*-type can be unambiguously identified with both acetolysed and hydrated methods, there are reasons why it might not be present as such in the result list of some samples. The sample itself might contain obstructions to the visibility of important pollen characteristics, making an identification down to a low taxonomic level unfeasible.

Similarly, pollen grains might be identified as *Brassica* sp. in one sample and Brassicaceae in another. A major characteristic of *Brassica* sp. grains in hydrated condition is the typical/average size of 26–28 µm in equatorial and/or polar diameter (see e.g. PalDat 2000 onwards; Stebler 2023) – a characteristic, which is not recommended to rely on in acetolysed samples, because pollen grains tend to slightly shrink during acetolysis (Halbritter et al. 2018; Koelzer et al. 2023). Hence, in acetolysed samples, they might be included in the Brassicaceae pollen type.

Although the multiple repetitions of a method generally produced comparable results, a distinct pollen type may be present in an unusually high abundance in one individual sample. For example, the high amount of *Tilia* sp. pollen (13%) in one of the acetolysed repetitions (1M_Muen_acet) may be due to a clump of *Tilia* sp. pollen grains in the portion of the sample that was analysed for this repetition. In hydrated samples, pollen grains of all types can potentially stick together. They may do so because of gluey pollenkitt, remaining sugar, prominent surface sculpturing, or as relic of the tetrad stage (Hesse 1981). In the acetolysed method, this is much rarer. Here, clumping may occur due to defects of the anther.

Choosing a preparation method

Both preparation methods have unique advantages and disadvantages when utilised for pollen analysis. While it is relatively quick and easy to prepare a hydrated sample, it is more time consuming and requires a laboratory equipped with necessary chemicals and fume hood to perform acetolysis (Halbritter et al. 2018). The availability of technical equipment including a light microscope with sufficient magnification (preferably a 60× objective lens for acetolysed samples and a 100× objective lens in oil for hydrated samples) and a digital camera is highly recommended for both methods. When using a smaller magnification, e.g. a 40× objective lens, certain characteristics of pollen grains cannot be seen and potential diagnostic information is lost.

For the analysis of a sample with high content of organic sediments such as yeasts, spores, algae, or parts of organisms, it may be beneficial to perform acetolysis for better visibility of pollen grains. Sometimes though, the presence or absence of certain non-pollen organic material can be important for the tracing of botanical and geographical origin as well as potential alterations and/or adulterations of honey. For example, to determine honeydew honey microscopically (Straka 1975; Beckh & Camps 2009), acetolysis would largely remove possible indicators.

One advantage of acetolysed pollen is the staining of the pollen wall, which increases the visibility of surface details (e.g. Lieux 1980; Halbritter et al. 2018), see Brassicaceae grain in Figure 7A. The staining of the pollen wall is darker, the more sporopollenin is contained in its structure, which usually corresponds to the thickness of the exine layer in the pollen wall, e.g. *Prunus* sp. (Figure 1I, J), *Tilia* sp. (Figure 2Q, R), *Parthenocissus* sp. (Figure 3I, J). After some months though, the brownish staining of the embedded acetolysed samples might fade, which should be considered especially when photographic documentation is desired. In hydrated samples, staining is not required but may be an asset. It can be achieved with agents such as fuchsine (aniline red), see the database Pollen-Wiki (Stebler 2023), and Figure 7C compared to the unstained pollen grain (Figure 7B). The dye can be mixed directly into the embedding material, as in Kaiser's glycerol gelatine (Mulisch & Welsch 2015). The quality of embedded hydrated samples will allow proper pollen type identification for years.

The choice of a preparation method might also affect the shape in which pollen grains appear. During acetolysis, the cytoplast and aperture mem- brane is destroyed, while in the hydrated state, the cytoplast usually swells. Some pollen types hence appear spheroidal when hydrated and prolate after acetolysis, e.g. *Robinia pseudoacacia* or *Trifolium*

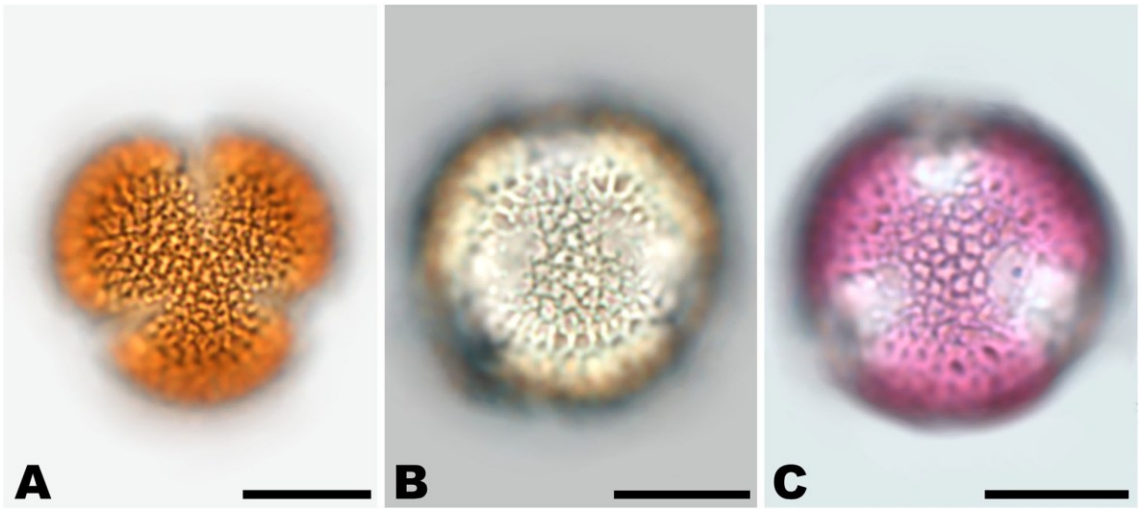

Figure 7. Brassicaceae pollen grain acetolysed (A), hydrated unstained (B) and hydrated stained with fuchsine (C). Scale bars – 10 µm. Source: Karen Koelzer.

repens-type (see Figure 4M–P). Therefore, relevant reference literature should be used for identification, preferably referring to the same method of preparation (acetolysed or hydrated). Pollen grains may also shrink to various extent during acetolysis. When using the size for identification purposes, measuring in the hydrated state is advised and allows to compare the measurements to the references in most online databases (PalDat 2000 onwards; Koelzer et al. 2023; Stebler 2023).

When applying the hydrated method, the use of 5 g of honey material will usually be sufficient for the analysis of 500 pollen grains on one slide, unless it is honey known to contain little amounts of pollen, such as for example unifloral *Robinia* honey (von der Ohe et al. 2004). With this volume, the pollen will also be spread out in a comfortable way in order to be able to identify and picture each grain individually while allow- ing for a continuous flow of counting.

The overall availability of literature should as well be considered when choosing a method. Literature can be limited in certain geographic regions and/or to certain methods. Gadbin (1979), who analysed honey from Chad Republic, could identify more pollen types acetolysed (15), compared to the hydrated method (12). He recommended acetolysis for honey from the central African region, firstly, because the pollen wall details are important for identification when the floral resources are not well known, and secondly, because of more available reference literature with the acetolysed method on the melliferous flora of the region. Similarly, Lieux (1980) recommended acetolysis for honey pollen analysis in the south-eastern United States, where the few palynologists at that time were trained in acetolysis. As one of the first honey researchers in the United States, she herself prepared more than 200 reference samples of foraging plants in Louisiana by using acetolysis (Lieux 1969). She also mentions the potential of shared reference literature with other areas of palynology (e.g. palaeobotany) in regions with less advanced palynological knowledge and limited resources (Lieux 1980). Countries, where acetolysis continues to be commonly used for the analysis of geographical and botanical origin are India (e.g. Bhargava et al. 2009; Ponnuchamy et al. 2014), and Nigeria (e.g. Durugbo et al. 2020; Ikegbunam et al. 2022). Plant species that are restricted to these regions may be easier to identify when using the same method likewise.

Lastly, the choice of preparation methods may also be considered in regard to potential further analyses with additional methods. With the cell content and intine dissolved, acetolysed pollen samples can still be processed for SEM (scanning electron microscopy) as well as TEM (transmission electron microscopy) analysis of the exine pollen wall. Unstained hydrated samples allow for more follow-up methods: iodine staining to detect starch and carmine staining to determine the number of nuclei (Halbritter et al. 2018), as well as electron microscopy for more detailed analyses of pollen wall structure/sculpture and cell organelles within the cytoplasm. In addition, hydrated samples can

potentially be further processed for DNA-analysis (as described e.g. in Bell et al. 2016). This might work best if the hydrated pollen sample is embedded in glycerine instead of glycerol gelatine, from which individual pollen grains may be extracted using a micromanipulator (Halbritter et al. 2018; Ulrich & Grímsson 2019).

For an elaborate comparison of acetolysed and hydrated methods, see Table II.

The human factor

Besides technical availability, pollen analyses may be unintentionally influenced by human errors. Possible factors of subjectivity may be the researcher's level of experience with using either the acetolysed or the hydrated method, the palynologist's condition and mood on the day of analysis, their confidence, time pressure through strict deadlines or limited time until closing hours, splitting the counting across several days, disruptions while counting, or increasing personal identification abilities within a row of multiple samples (e.g. Galán et al. 2014). However, colour vision deficiencies, such as described for pollen pellet sorting in Hornby et al. (2022), are a neglectable factor in microscopic analyses, since pollen grains are identified with their more prominent structural and sculptural characteristics rather than solely by colour.

As a self-critical approach to test for potential other biases than preparation methods during counting out pollen samples, the second part of this study included multiple repetitive analyses of the same honey. Differences between the acetolysed and hydrated method were reproducible in regards to pollen diversity as well as specific taxa (e.g. *Myosotis* sp.). Differences within the acetolysed and the hydrated group,

Table II. Side-by-side comparison of multiple factors with the acetolysed and hydrated method

		Method	
		Acetolysed	Hydrated
Laboratory work	Required amount of honey material[1]	Minimum 10 g	Minimum 5 g
	Age and condition of the honey	Fresh to subfossil	Fresh, up to 5 years before cytoplast slowly disintegrates
	Required chemicals and laboratory equipment	Deionised water, acetic acid, acetic anhydride, concentrated sulphuric acid, ultrasonic water bath, fume hood, centrifuge, plunger-operated pipette	Deionised water, centrifuge, heating plate, plunger-operated pipette
	Preparation time	90 min	30 min
	Resting time after embedding on object slide	15 min (glycerine) or longer/overnight	1 day (glycerol gelatine)
	Optimal LM magnification (optical lens)	60×	100× in oil
Identification tools for LM	Reference literature	Available, abundant	Available
	Reference online databases	Available	Available, abundant
Pollen characteristics	Visibility of pollen grains in the sample	Excellent	May be reduced by debris such as starch globules, yeasts, spores, algae, bee parts and wax tubes
	Staining of pollen grains	Brownish due to acetolysis	Natural colour of the pollen grains
	Pollen wall details	Highlighted through staining, no obstructions	Might be hidden by obstructions
	Pollen surface	Usually clean	Can be obstructed by sugar remains, other organic content, or pollenkitt
	Ornamentation of aperture membrane	May be lost	Usually intact
	Aperture seams	Usually clearly visible	Not always clearly visible
	Cytoplast at aperture	Destroyed	Appears in taxa-typical way
	Cytoplast contents	Destroyed	Potentially contains starch and/or lipid globules
Further handling	Sample availability for other methods	SEM, TEM	Staining with iodine or carmine, acetolysis, SEM, TEM, DNA-barcoding

respectively, were neglectable and did not have an effect on the overall integrity of results. The number of identified pollen taxa and the percentages of the most important pollen types were comparable.

Relevance of LM analysis in times of DNA-metabarcoding

Our study shows that both the acetolysed and the hydrated methods are technically suitable for the qualitative and quantitative analyses of pollen in honey. Despite the larger pollen spectrum identified with the hydrated method, there are still limits to taxonomic resolution and the number of identified taxa. Such limitations may be overcome by promising new methods such as DNA metabarcoding. DNA metabarcoding has been used to analyse and quantify the full pollen spectrum in honey (e.g. Smart et al. 2017; Noël et al. 2023). These studies showed strong, yet taxa-specific, concordance to LM results. DNA metabarcoding may complement LM methods as it potentially enables a higher taxonomic resolution for pollen types that can only be identified collectively to family level by LM, such as Poaceae, Pinaceae, or Cupressaceae (Leontidou et al. 2021). Moreover, it was shown to surpass LM when it comes to taxa richness, especially when taxa were present in little abundances (Smart et al. 2017; Polling et al. 2022). However, limitations concerning the reliable identification of certain plant taxa have been observed with DNA metabarcoding as well. These are mostly due to the choice of primer system in combination with the available reference databases. Another limitation is the quantification of pollen abundances. Some studies have quantitatively compared the relative number of amplicon reads from DNA metabarcoding to that of microscopic pollen counts. Effects of pollen morphology (Swenson & Gemeinholzer 2021) and amplification biases through primer mismatch (Bänsch et al. 2020; Leontidou et al. 2021; Polling et al. 2022) may be a common cause for large proportions of unexplained abundance variations. Hence, at the current state of knowledge, verification by LM and botanical plausibility checks are vital to detect false positive results at the stage of taxon assignment. Due to the current technical limitations observed in DNA metabarcoding, it cannot fully replace LM analyses. Here, especially the hydrated method remains the method of choice for reliable and, in general, reproducible quantitative melissopalynological data.

Conclusions

Both the hydrated and acetolysed methods of preparation for the pollen analysis of honey have their unique advantages. In order to identify pollen types correctly applying either method, it needs a high degree of experience, access to reference literature, and awareness of the limitations. For most uses and quality requirements, analysing the sample with either method (acetolysed/hydrated) is sufficient. The reproducibility could be shown through multiple repetitions with acetolysed as well as hydrated material. Remarkably, the hydrated method revealed an overall higher number of taxa on the object slide in total and within 500 counted pollen grains.

For experienced palynologists, pollen analysis of a honey sample takes less than a day of time, from preparation to the list of results. LM methods, especially hydrated samples in glycerol gelatine, require a minimum of costs and may be wider accessible for researchers, students and interested apiarists alike, compared to acetolysis and newer techniques such as DNA metabarcoding.

For going from experimental research to the implementation of novel methods in routine analysis on the official scale, further extensive validation of methods is essential. Here, LM (acetolysis or hydration) functions as a backbone for method development and needs to be conducted with diligence and sufficient photographic documentation. So far, melissopalynology needs the LM techniques and cannot be fully replaced by DNA-methods. However, the latter could be a very useful addition to future research.

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Supplemental data

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C. TREES ARE A MAJOR FORAGING RESOURCE FOR HONEYBEES IN THE CITY

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Trees Are a Major Foraging Resource for Honeybees in the City

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Abstract: Large cities are typically characterized by a mosaic of green spaces that hold a remarkable variety of native and "exotic" plants. Urban beekeeping has gained increasing popularity. In order to characterize the "urban" in the honey, pollen diversity in 50 honey samples from 18 apiary locations in Vienna, Austria, was microscopically analyzed. The relative abundances of each plant taxon were determined by counting out 500 individual pollen grains per sample. In total, 202 taxa could be identified, with a median of 46 per sample. Taxa richness and diversity differed significantly across three years but did not so between urban and suburban apiaries. Despite trees comprising only roughly a quarter of all taxa, the amount of tree pollen was disproportionally high. The invasive *Ailanthus altissima* was predominant in 15 out of 50 samples. Other important non-native and/or ornamental trees included *Sophora japonica*, *Gleditsia triacanthos*, *Castanea sativa*, *Koelreuteria paniculata*, and *Liriodendron tulipifera*. Urban honey from Central Europe may typically comprise pollen taxa from Europe, East Asia, and North America alike. The results of this study show that intentionally planted, managed urban green spaces can support stable foraging resources for pollinators in cities.

Keywords: floral resources; honey; melissopalynology; plant diversity; pollen analysis; pollinators; urban ecology

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1. Introduction

Urban landscapes are characterized by the heterogeneity of floral resources and habitats [1,2]. However, the high proportion of sealed surfaces is an inhospitable matrix for many pollinators. In addition, exposure to higher temperatures in "urban heat islands" affects not only the physiology of plant species but also the quality of plant–pollinator interactions [3]. Worldwide, a decline in pollinators could be linked to human pressures, environmental change, and a high degree of fragmentation [4,5].

Plants in the city are vital for humans and animals in many aspects. In view of global warming, trees, in particular, have become an important source of shade for residents, besides fulfilling an aesthetic purpose. At the same time, they act as a valuable food source for pollinating insects. Appropriately managed urban green spaces can act as hotspots for pollination services, specifically for bees [6,7].

Honeybees of the *Apis mellifera* subsp. *carnica* race, which is the most frequently kept honeybee type in Austria [8], are generalist flower visitors. They collect pollen and store it inside the hive as beebread (perga), which provides the larvae with protein. Nectar is stored in the comb cells as honey and is the colony's primary source of sugar. Pollen grains are indirectly introduced to honey through secondary input in the beehive [9,10]. The specific

morphology and characteristics of pollen grains allow us to microscopically identify their taxonomic source and hence link the honey to the botanical and geographical source of honeybee foraging [11,12].

Urban beekeeping has many benefits: It contributes to a healthy environment by maintaining plant–pollinator interaction in urban areas, provides economic profits for beekeepers and apiary localities, and is a valuable tool to educate the general public about the importance of pollinators [13,14]. There is a continuous trend to keep honeybees in urban environments. Yet, the authenticity of urban honey from a melissopalynological perspective is often unclear.

Urban landscapes present particular challenges for melissopalynological pollen analyses due to a high diversity of both native and ornamental plants [15]. Ornamentals, especially, have become a vital part of municipal and private planting regimes [16]. Typically, certain "marker" pollen types indicate the origin of a honey sample by knowing the geographical distribution of that plant taxon. As relatively few plant individuals can provide vast amounts of flowers, trees especially comprise an important food source for bees in disturbed areas such as urban environments [17,18]. In recent years, however, tree species from other geographical regions have been widely introduced to Central European cities as inner-city environments increasingly mimic the heat and drought conditions of their original distributions. Those trees include, e.g., *Ailanthus altissima*, *Koelreuteria paniculata*, and *Sophora japonica* from the Qinling Mountains in China, *Gleditsia triacanthos* and *Liriodendron tulipifera* from the Appalachians in the United States, and *Tilia tomentosa* from the East European steppes [19,20]. When available in higher abundances, introduced and non-native plants may also change the seasonal pattern of foraging resources for pollinators [3].

A more systematic approach is essential to shed knowledge on the spatial and temporal availability of floral resources in cities and to understand honeybee preferences. The aim of this study is to gain basic knowledge of the actual foraging resources in a Central European urban metropolis and highlight the importance of planted trees for honeybees. It examines the availability and origin of trees across the heterogeneous mosaic of urban and suburban landscapes. Knowing how honeybees interact with the floral resources in their environment will provide information for the management of municipal planting regimes, as well as point private owners of gardens towards honeybee-friendly plant choices. Moreover, this study will give scientific guidance for beekeepers regarding the spatial and temporal availability of floral resources in a city and how they might impact hive management.

2. Results

For this study, 50 honey samples were melissopalynologically analyzed. This includes nineteen honey samples in 2020, twenty samples in 2021, and eleven samples in 2022. Across all samples and all three years, a total of 202 individual pollen types could be identified. Out of these, 52 pollen types could be identified at the species level (25.7%), 91 at the genus level (45%), and 23 at the family level (11.4%). A further 5 could be identified down to two or three potential genera (2.5%), 25 were classified as "type" (12.4%), and 6 remained unidentified (3%). Across all years, the mean number of taxa per sample was 46. The mean number of taxa was highest in 2022 (Figure 1A). In general, the number of identified plant taxa did not correlate with the number of apiary locations per year (see Table 1).

Mean taxa richness and mean Shannon Diversity Index were calculated for the different years (Figure 1A,D), the urban and suburban groups (Figure 1B,E), and the different harvesting times (Figure 1C,F). The diversity of foraged plants was significantly different between the years (Table 1) but did not significantly change from urban to suburban locations and between extraction times. Differences in taxa composition were assessed by permutational multivariate analysis of variance, which revealed significant differences by year, urban vs. suburban location, and extraction time.

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Table 1. Number of samples, apiary locations, and identified taxa per year.

Year	*n* Samples	*n* Locations	*n* Taxa
2020	19	15	150
2021	20	16	141
2022	11	11	142

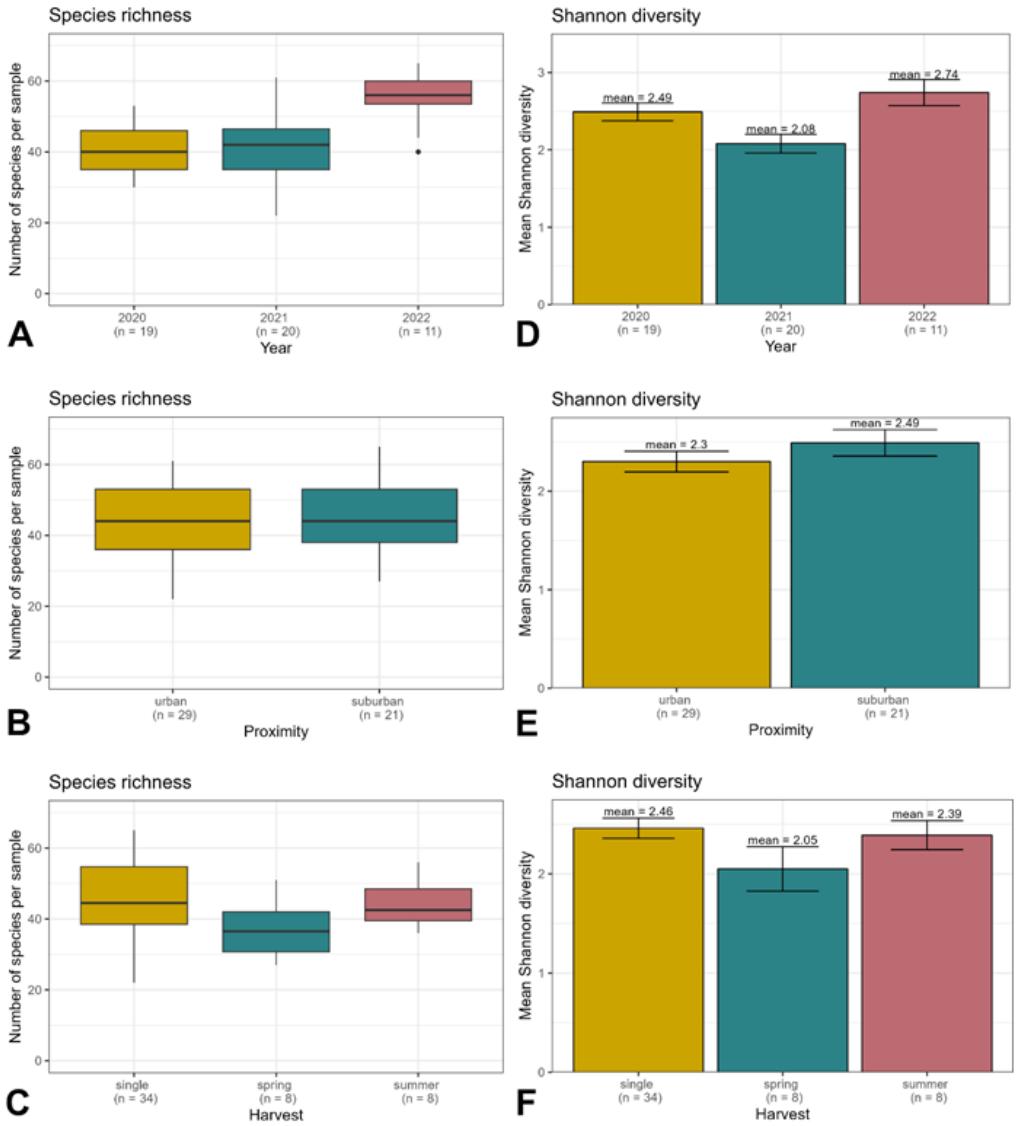

Figure 1. (**A**–**C**): Taxa richness per sample across three years (2020–2022; (**A**), by proximity (urban and suburban; (**B**), and three harvesting times (single harvest in June, spring harvest in the first half of July, and summer harvest in July–August; (**C**). (**D**–**F**) Mean Shannon Diversity Index of pollen spectra across years (**D**), proximity (**E**), and harvesting times (**F**).

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Further, a growth type was assigned to each of the 202 plant taxa, if possible. As shown in Figure 2A, they belong to herbaceous species (108), trees (49), shrubs (28), lianas (3), epiphytes (2), and various growth types (12). The number of tree taxa was clearly lower than the number of herbaceous species. Despite that, the relative abundance of tree pollen per 500 counted pollen grains by far exceeded all other categories (Figure 2B), with a median of 350. This trend was reproducible in 2020 and 2021 across all three harvesting times (single harvest, spring harvest, and summer harvest); see Figure 2C,D.

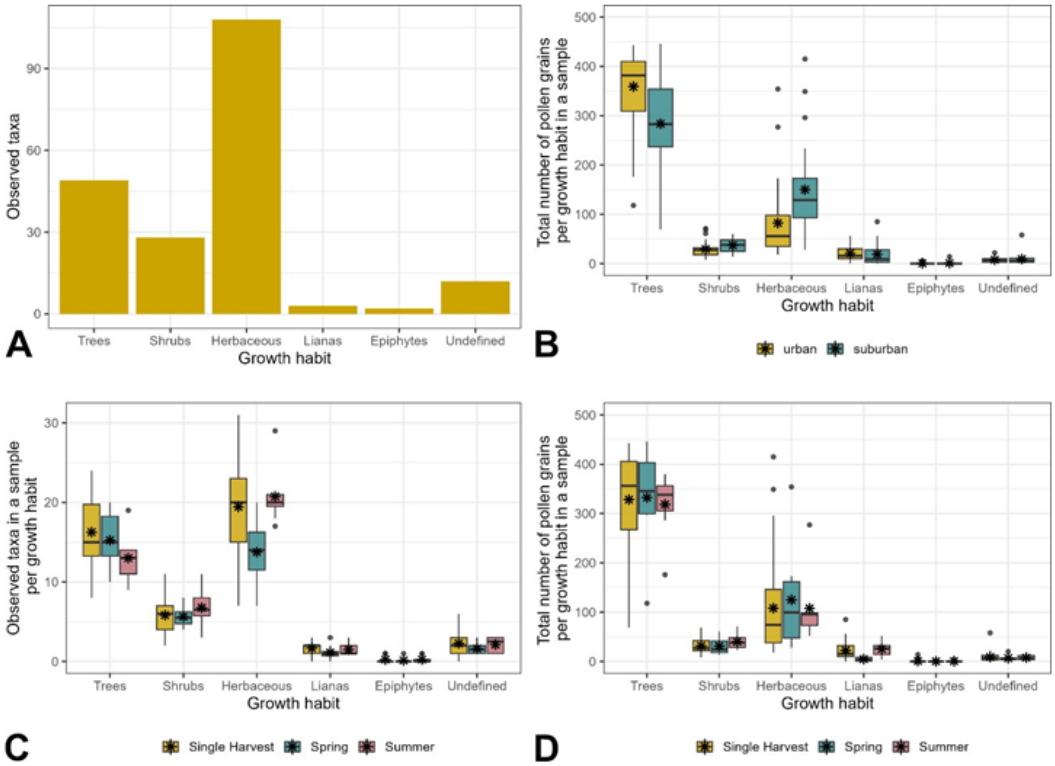

Figure 2. Number of identified taxa per growth habit (**A**), total amount of pollen per growth habit per 500 counted grains in the urban and suburban groups (**B**), distinct taxa counts per growth habit at different harvesting times (**C**), total amount of pollen grains per growth habit per 500 counted pollen grains at different harvesting times (**D**). Asterisks indicate the mean value across all 50 honey samples.

To determine the overall most abundant taxa, all pollen types with more than 8% relative abundance in at least one sample were considered (Figure 3). Out of these 23 taxa, ten belonged to trees. They were *Acer* sp., *Aesculus hippocastanum*, *Ailanthus altissima*, *Castanea sativa*, *Gleditsia triacanthos*, *Malus*-Type, *Prunus* sp., *Salix* sp., *Sophora japonica*, and *Tilia* species. Two were shrubs (*Rubus* sp. and *Syringa* sp.), and two were lianas (*Parthenocissus* sp. and *Vitis vinifera*). The rest was herbaceous or of various growth types. Only five identified pollen types occurred predominantly (>45%) in at least one honey sample. These were *Ailanthus altissima*, *Castanea sativa*, *Myosotis* sp., *Sophora japonica*, and *Brassica* species. Micrographs of the most occurring tree taxa are shown in Figures 4–6, and of the most occurring shrubs in Figure 7.

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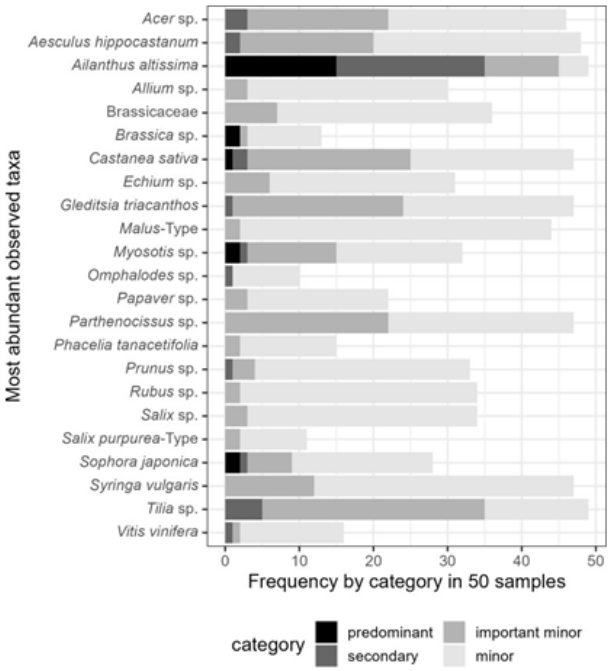

Figure 3. Pollen types with more than 8% abundance in at least one of the 50 honey samples. The occurrence is displayed according to defined frequency categories (predominant = > 45%, secondary = 15–45%, important minor = 3–15%, minor = < 3%).

The seven taxa most widely occurring across all 50 honey samples (see Table 2) were *Ailanthus altissima*, *Tilia* sp., *Aesculus hippocastanum*, *Gleditsia triacanthos*, *Parthenocissus* sp., *Syringa* sp., and *Castanea sativa*. *Ailanthus altissima* pollen (Figure 4A,B) occurred in 49 out of 50 honey samples and at all 18 apiary locations. Its highest relative abundance per 500 counted pollen grains was 80.6%, with a mean of 31%. It was the predominant pollen type (>45%) in 15 out of 50 samples. Pollen of *Tilia* sp. was likewise present in 49 out of 50 honeys and at all 18 apiary locations but was predominant in neither sample. The relative abundance of *Tilia* sp. pollen grains was highest at 37.2%, with a mean of 6.6%.

To examine the importance of non-native and/or ornamental species, the identified taxa were assigned to one or more of the following four categories: established/native to Austria, invasive in Austria, ornamental species, and agricultural species. The majority of taxa were native/established (145), followed by ornamental (71), agricultural (13), and invasive species (12). *Ailanthus altissima*, for example, was included in the established and invasive categories.

Out of the 49 identified tree taxa, 19 were non-native ornamentals (see Table 3). Those occurring in more than half of all analyzed honey samples and locations were *Sophora japonica* (Figure 4C,D), *Gleditsia triacanthos* (Figure 4I,J), *Koelreuteria paniculata* (Figure 5A,B), and *Liriodendron tulipifera* (Figure 5K,L).

Pollen of nectarless species was included to cover the full diversity of botanical origin and occurred in all 50 honey samples. Neither the apiary location in urban or suburban areas nor the harvesting time did affect the abundance of nectarless taxa significantly. The most frequent pollen of nectarless trees was of *Betula* sp. (Figure 6O,P), *Morus* sp. (Figure 5M,N), *Pinus* sp., *Platanus* sp. (Figure 6E,F), *Quercus* sp. (Figure 6A,B), and *Taxus* sp. (Figure 5O,P). None of these taxa occurred with more than 3% relative abundance in any of the samples.

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Table 2. The 33 pollen types occurring in more than 50% of all analyzed samples, their growth habit (T = tree, S = shrub, H = herbaceous, L = liana, E = epiphyte), number of samples, number of samples per frequency category (predominant = > 45%, secondary = 15–45%, important minor = 3–15%, minor = < 3%), and maximum relative abundance in %.

Taxa Name	Family	Growth Type	*n* Samples	> 45%	15–45%	3–15%	<3%	Max. (%)
Ailanthus altissima	Simaroubaceae	T	49	15	20	10	4	80.60
Tilia sp.	Malvaceae	T	49	0	5	30	14	37.20
Aesculus hippocastanum	Sapindaceae	T	48	0	2	18	28	30
Castanea sativa	Fagaceae	T	47	1	2	22	22	49
Gleditsia triacanthos	Fabaceae	T	47	0	1	23	23	39.60
Parthenocissus sp.	Vitaceae	L	47	0	0	22	25	11.20
Syringa sp.	Oleaceae	S	47	0	0	12	35	8.20
Acer sp.	Sapindaceae	T	46	0	3	19	24	19.20
Plantago sp.	Plantaginaceae	H	46	0	0	1	45	3.60
Trifolium repens-Type	Fabaceae	H	46	0	0	4	42	6.80
Sambucus-Type	NA	S	46	0	0	9	37	5.20
Malus-Type	Rosaceae	T	44	0	0	2	42	11.80
unidentified	unidentified	NA	43	0	0	0	43	2.20
Anemone-Type	Ranunculaceae	H	39	0	0	1	38	3.20
Koelreuteria paniculata	Sapindaceae	T	38	0	0	5	33	4.20
Poaceae	Poaceae	H	37	0	0	1	36	3
Brassicaceae	Brassicaceae	H	36	0	0	7	29	13.80
Cornus sanguinea	Cornaceae	S	36	0	0	1	35	5.60
Salix sp.	Salicaceae	T	34	0	0	3	31	9.60
Rubus sp.	Rosaceae	S	34	0	0	2	32	9.60
Prunus sp.	Rosaceae	T	33	0	1	3	29	31.80
Myosotis sp.	Boraginaceae	H	32	2	1	12	17	64.80
Fragaria sp./*Potentilla* sp.	Rosaceae	H	32	0	0	1	31	4
Echium sp.	Boraginaceae	H	31	0	0	6	25	9.20
Allium sp.	Alliaceae	H	30	0	0	3	27	10.80
Buddleja (4-aperturate)	Scrophulariaceae	S	29	0	0	3	26	6.40
Sophora japonica	Fabaceae	T	28	2	1	6	19	63
Taraxacum-Type	Asteraceae	H	28	0	0	0	28	1
Begonia sp.	Begoniaceae	H	28	0	0	1	27	3
Robinia pseudoacacia	Fabaceae	T	27	0	0	0	27	1.60
Rosaceae	Rosaceae	NA	26	0	0	0	26	2
Lamiaceae (6-aperturate)	Lamiaceae	H	25	0	0	0	25	0.60
Liriodendron tulipifera	Magnoliaceae	T	25	0	0	0	25	1

Table 3. Pollen of non-native ornamental tree species (19) and their biogeographical origin, as well as the number of samples in which they occurred, the maximum relative abundance (max. %), and whether the plants produce nectar.

Taxa Name	Family	Origin	*n* Samples	Max. (%)	Nectar
Catalpa sp.	Bignoniaceae	North America, East Asia	8	0.4	yes
Celtis sp.	Cannabaceae	Southern Europe, North America	1	0.2	no
Corylus colurna	Betulaceae	South-Eastern Europe, Asia	6	0.6	no
Davidia involucrata	Nyssaceae	East Asia	1	1	yes
Fraxinus ornus	Oleaceae	Mediterranean	20	1.8	yes
Ginkgo biloba	Ginkgoaceae	East Asia	4	0.4	no
Gleditia triacanthos	Fabaceae	North America	47	39.6	yes
Koelreuteria paniculata	Sapindaceae	East Asia	38	4.2	yes
Liquidambar styraciflua	Altingiaceae	North America	1	0.2	yes
Liriodendron tulipifera	Magnoliaceae	North America	25	1	yes
Morus sp.	Moraceae	South-Eastern Europe	11	2	no
Paulownia tomentosa	Paulowniaceae	East Asia	2	1	yes
Platanus sp.	Platanaceae	Southern Europe, Asia	20	1	no
Pterocarya fraxinifolia	Juglandaceae	Caucasus	2	0.2	no
Ptelea trifoliata	Rutaceae	North America	1	0.2	yes
Sophora japonica	Fabaceae	East Asia	28	63	yes
Tamarix sp.	Tamaricaceae	Southern Europe, Asia, Africa	3	5.6	yes
Tetradium daniellii	Rutaceae	East Asia	12	1.2	yes
Zelkova serrata	Ulmaceae	East Asia	1	0.4	no

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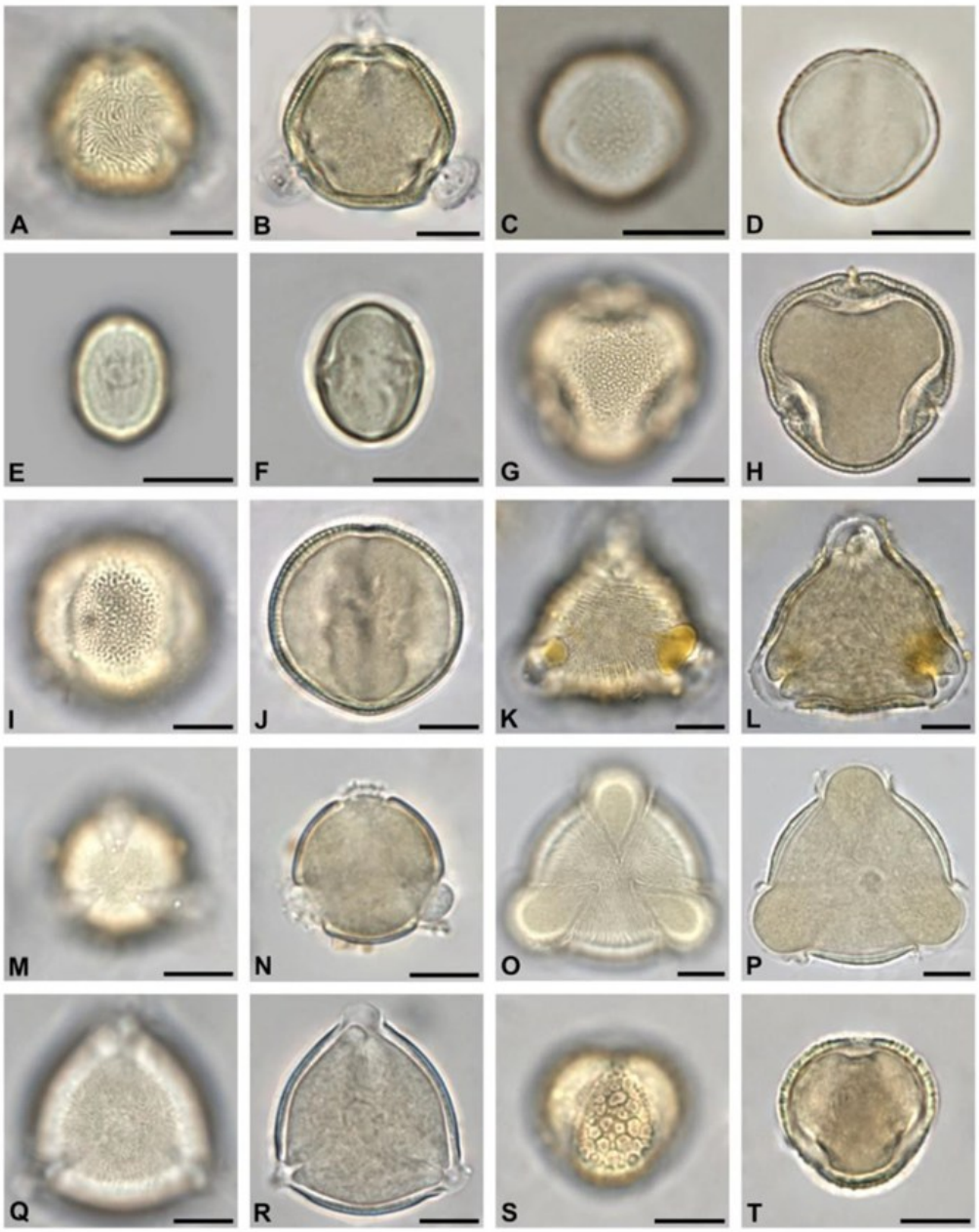

Figure 4. The thirty most abundant tree pollen types in honey from Vienna, Part I: *Ailanthus altissima* in polar view (**A**,**B**), *Sophora japonica* in equatorial view (**C**,**D**), *Castanea sativa* in equatorial view (**E**,**F**), *Tilia* sp. in polar view (**G**,**H**), *Gleditsia triacanthos* in equatorial view (**I**,**J**), *Prunus* sp. in polar view (**K**,**L**), *Aesculus hippocastanum* in polar view (**M**,**N**), *Acer* sp. in polar view (**O**,**P**), *Malus*-Type in polar view (**Q**,**R**), and *Salix* sp. in equatorial view (**S**,**T**). Scale bar indicates 10 µm.

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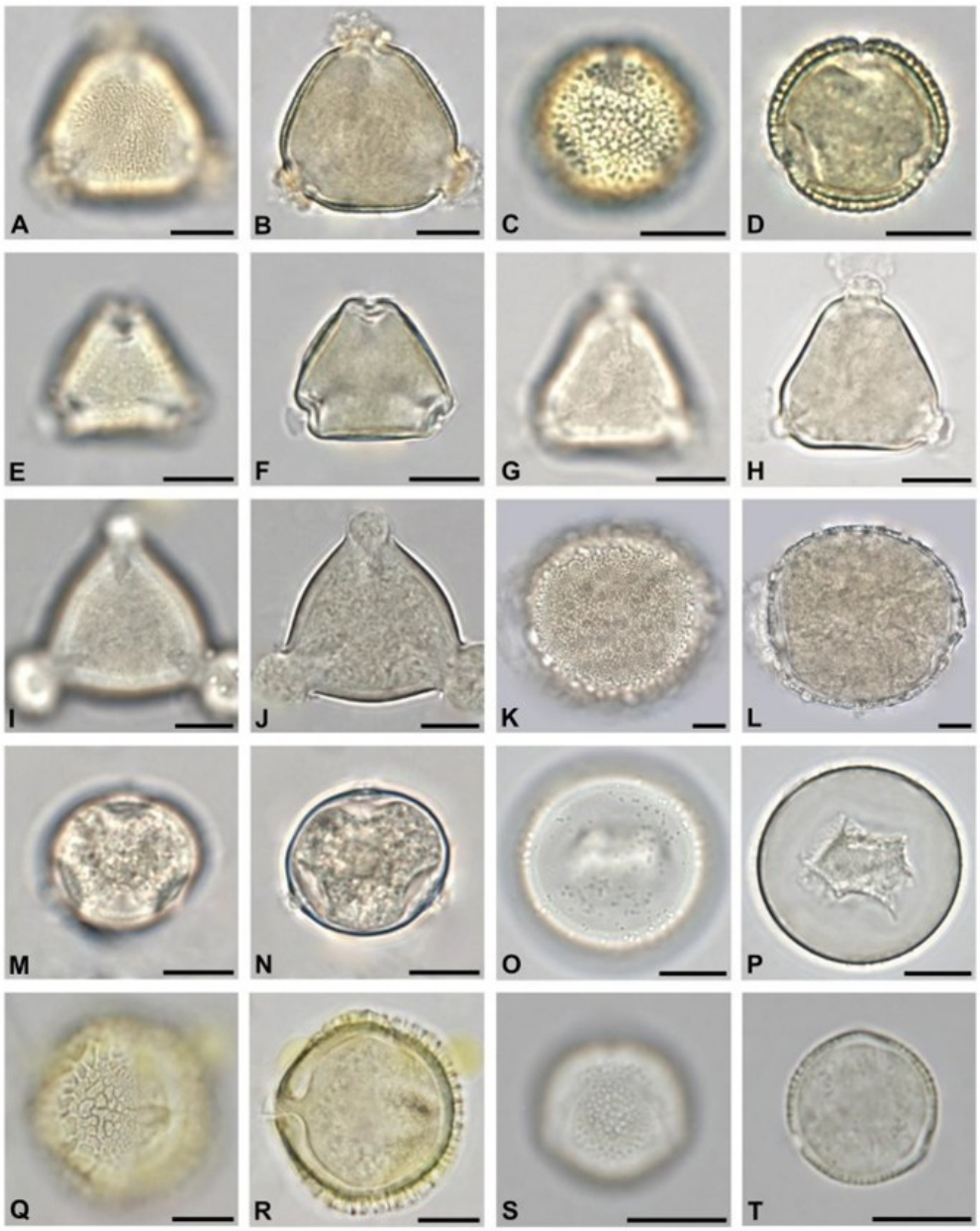

Figure 5. The thirty most abundant tree pollen types in honey from Vienna, Part II: *Koelreuteria paniculata* in polar view (**A**,**B**), *Fraxinus ornus* in polar view (**C**,**D**), *Rhamnus* sp. in polar view (**E**,**F**), *Frangula* sp. in polar view (**G**,**H**), *Robinia pseudoacacia* in polar view (**I**,**J**), *Liriodendron tulipifera* in polar view (**K**,**L**), *Morus* sp. in polar view (**M**,**N**), *Taxus* sp. (**O**,**P**), *Tetradium daniellii* in equatorial view (**Q**,**R**), and *Tamarix* sp. in equatorial view (**S**,**T**). Scale bar indicates 10 µm.

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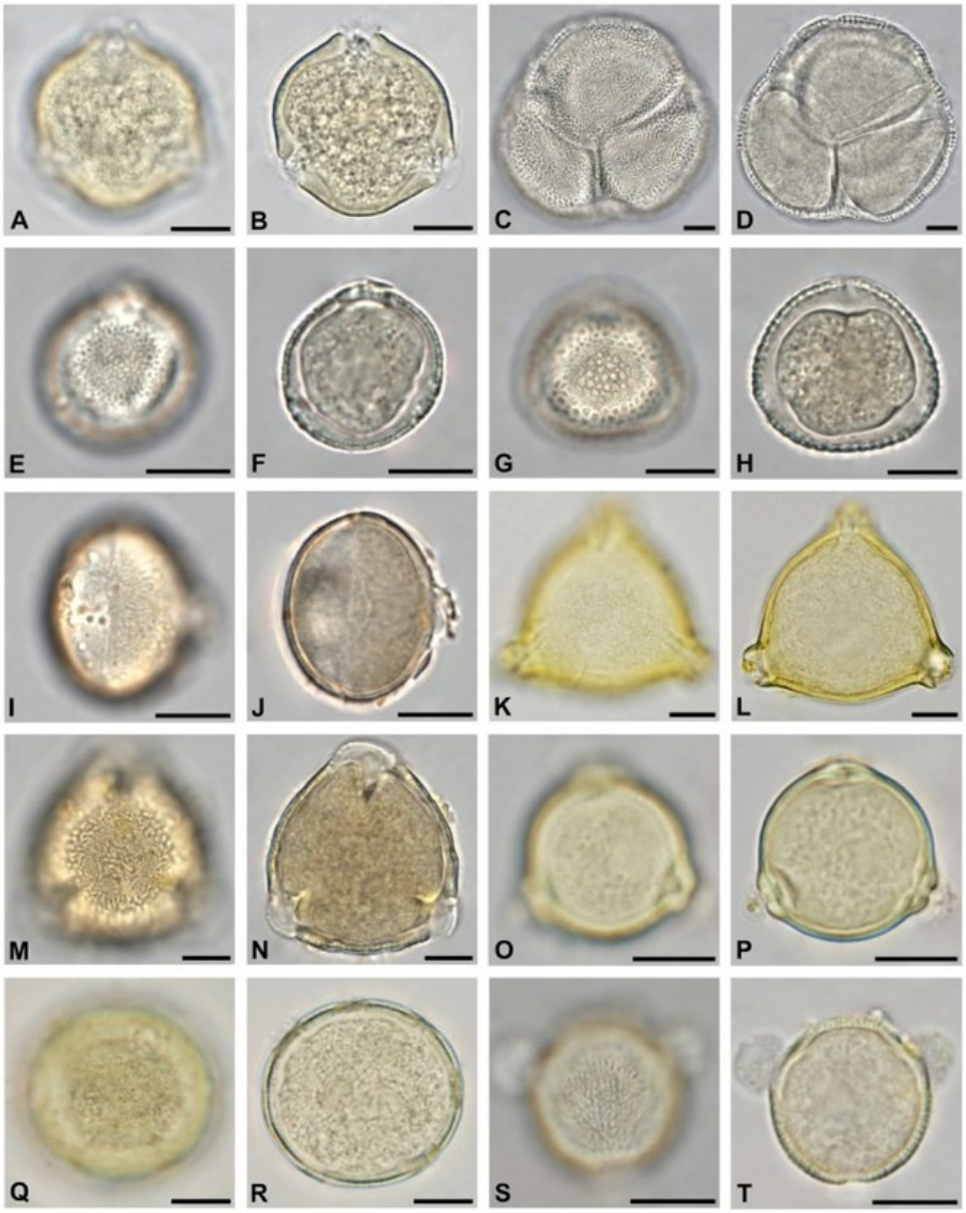

Figure 6. The thirty most abundant tree pollen types in honey from Vienna, Part III: *Quercus* sp. in polar view (**A**,**B**), tetrad of *Catalpa* sp. (**C**,**D**), *Platanus* sp. in polar view (**E**,**F**), *Fraxinus excelsior* in polar view (**G**,**H**), *Aesculus* x *carnea* in equatorial view (**I**,**J**), *Elaeagnus angustifolia* in polar view (**K**,**L**), *Prunus domestica* in polar view (**M**,**N**), *Betula* sp. in polar view (**O**,**P**), *Liquidambar styraciflua* (**Q**,**R**), and *Cotinus coggygria* in polar view (**S**,**T**). Scale bar indicates 10 µm.

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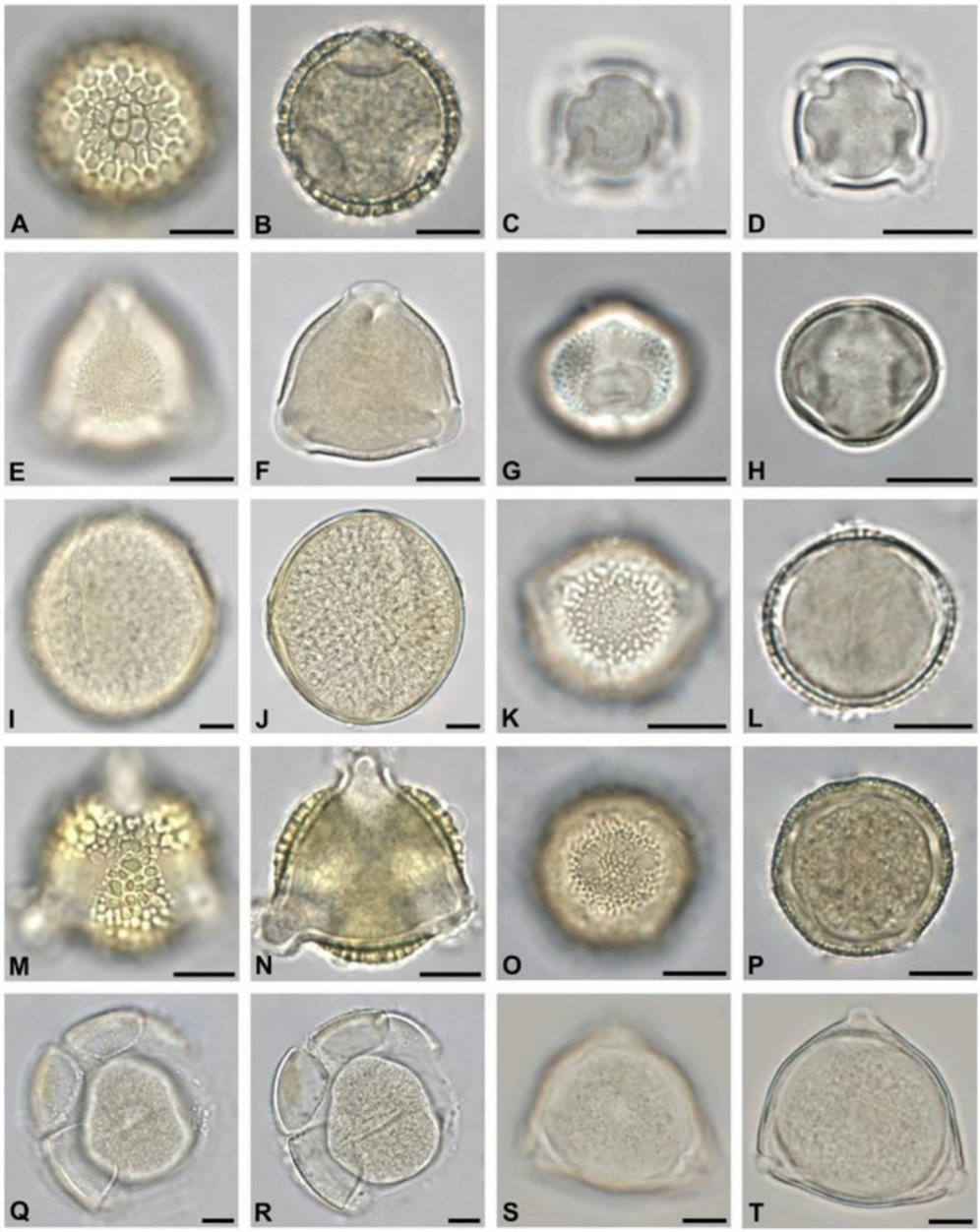

Figure 7. The ten most abundant pollen types of shrubs in honey from Vienna: *Syringa* sp. in polar view (**A**,**B**), *Buddleja* sp. in polar view (**C**,**D**), *Rubus* sp. in polar view (**E**,**F**), *Sambucus*-Type in equatorial view (**G**,**H**), *Cornus sanguinea* in equatorial view (**I**,**J**), *Viburnum lantana*-Type in equatorial view (**K**,**L**), *Ilex aquifolium* in polar view (**M**,**N**), *Buxus sempervirens* (**O**,**P**), *Berberis* sp. (**Q**,**R**), and *Symphoricarpos* sp. in polar view (**S**,**T**). Scale bar indicates 10 µm.

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2.1. Urban and Suburban Sites

According to the proximity to the city center and the amount of dense grayscape within the 3 km flight radius, 29 honey samples were classified as from urban locations and 21 as from suburban locations (Table 4). In the "urban" group, 167 out of 202 pollen types were observed, while the "suburban" group contained 164 out of 202 pollen types. The mean number of taxa per sample was similar between the two groups (Figure 1B). Taxa richness between the urban and suburban groups differed non-significantly across the years, with the largest variation in 2021.

Table 4. List of apiaries with the number of analyzed samples per year and their surroundings classified as urban and suburban, respectively.

Sample	District	Location	2020	2021	2022	Proximity
25	1st	Stubenring	1	1	1	Urban
31	3rd	Hotel Daniel	2	2	-	Urban
44	4th	Technical University of Vienna	-	1	1	Urban
39	6th	ibis Hotel	1	1	-	Urban
26	8th	Palais Auersperg	1	1	1	Urban
36	10th	Suchenwirtplatz	1	1	1	Urban
27	12th	Eichenstraße	1	1	1	Urban
28	15th a	Boutiquehotel Stadthalle	1	1	1	Urban
32	15th b	KGV Schmelz	2	2	-	Urban
46	20th	Donaukanal	-	1	1	Urban
33	2nd	Prater Lusthaus	2	2	-	Suburban
45	11th	Gärtnerei Auer	-	1	1	Suburban
40	14th	Flötzersteig	1	2	-	Suburban
29	18th	University of Natural Resources and Life Sciences	1	1	1	Suburban
59	21st	An der Schanze	1	-	-	Suburban
30	22nd	NH Danube City Hotel	1	-	1	Suburban
34	23rd	Pappelteich	2	1	-	Suburban
37	AP	Vienna International Airport	1	1	1	Suburban

The amount of tree pollen with respect to the number of tree taxa was disproportionally high in urban as well as suburban sites (Figure 2A,B). The median number of pollen from herbaceous species per sample was considerably higher in suburban areas than in urban areas (Figure 2B).

The number of ornamental plant taxa, as well as the relative abundances of ornamentals' pollen per 500 counted grains, was higher in urban sites closer to the city center; however, the differences were not statistically significant.

2.2. Time and Frequency of Honey Extraction

In the years 2020 and 2021, honey from four locations was extracted twice. The spring harvest (indicated by "1" in Figures 1 and 2) took place in the first half of June. The summer harvest (indicated by "2") followed at the end of July. All other honey samples were derived from a single harvest in the first half of July (indicated by "0"). General taxa richness was higher in later extraction times compared to honey harvested in spring (Figure 1C). The mean number of identified tree taxa per sample, however, decreased from 15 in the first harvest to 13 in the second harvest (Figure 2C). The least number of herbaceous taxa occurred in spring. At all harvesting times, the relative abundances of tree pollen were disproportionally higher compared to the number of tree taxa (Figure 2C,D). The frequency of tree pollen per 500 pollen grains was similarly high in honey from spring, summer, and a single harvest.

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2.3. Comparison of Four Different Locations Across Three Years

Four apiary locations were selected for a more detailed look at pollen diversities, as they represent the gradient from densely built-on urban areas to more open suburban landscapes. They were in the 1st and 15th districts of Vienna, as well as the 18th district and the airport (Figure 8).

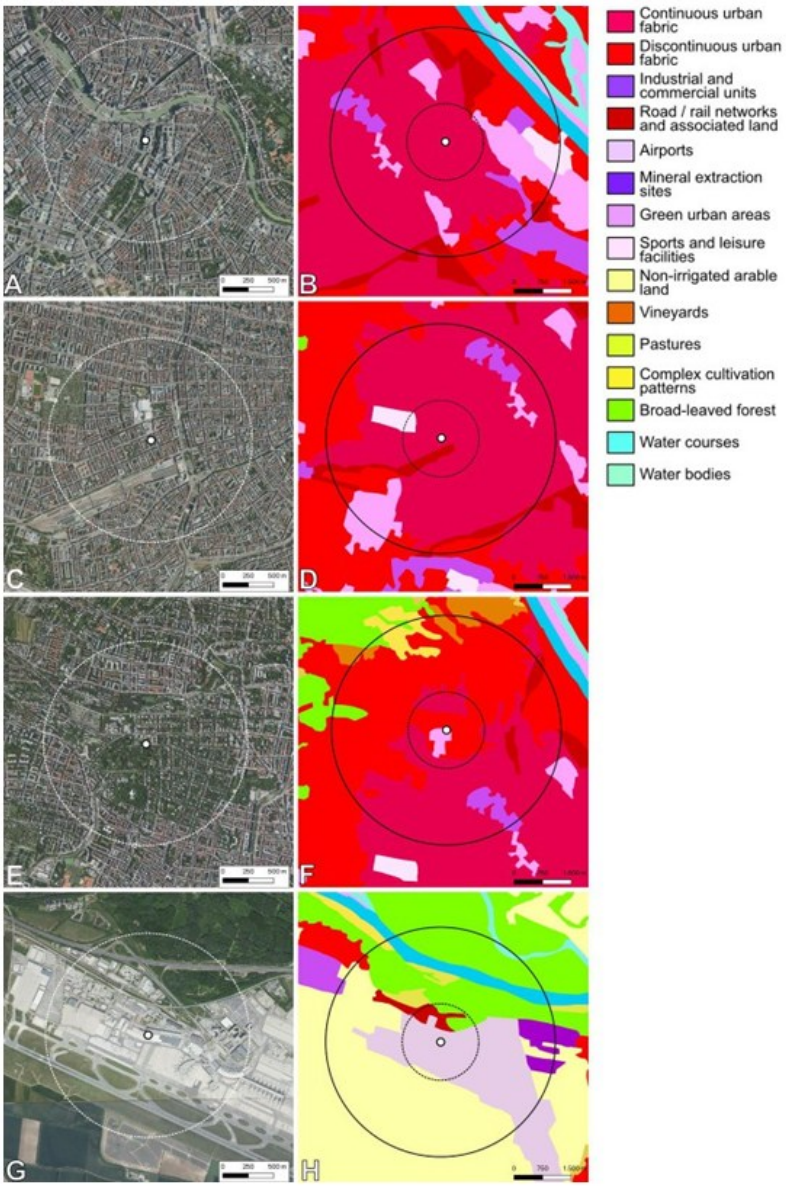

Figure 8. Selected apiary locations and the surrounding landscapes as ortho-photo with a 1 km radius (left), as well as the CORINE land cover analysis with a 1 km and 3 km radius (right): 1st district Stubenring (**A**,**B**), 15th district Boutiquehotel Stadthalle (**C**,**D**), 18th district University of Natural Resources and Life Sciences (**E**,**F**), and Vienna International Airport (**G**,**H**).

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The amounts of tree pollen within 500 counted pollen grains were highest at the city center and decreased towards the outskirts. At all locations except for the airport, pollen of trees occurred in disproportionally high amounts (see Figure 9).

The mean number of identified pollen types across all three years was highest in the 18th district (51) and lowest in the 1st district (40). The highest number of tree taxa occurred in the 18th district in the year 2022 (24). The least number of tree taxa were identified in honey from the airport, with only eleven taxa in the year 2020. Similarly, the relative abundance of tree pollen in honey from the airport was low, with a minimum of 13.8% in the year 2022. This was the only sample out of 50 where the amount of counted tree pollen was negatively correlated to the number of tree taxa.

The most frequent tree pollen types in the 1st and 15th districts were *Ailanthus altissima* and *Tilia* species. They occurred, among others, together with *Acer* sp., *Aesculus hippocastanum*, *Gleditsia triacanthos*, and *Koelreuteria paniculata*. Towards the outskirts, the amounts of *Ailanthus altissima* and *Tilia* pollen decreased and the amount of *Castanea sativa*, *Malus*-Type, *Prunus* sp., and *Salix* pollen increased. The most frequent herbaceous species at the four selected locations were Brassicaceae, *Myosotis* sp., *Echium* sp., and *Phacelia tanacetifolia*. In addition, a high frequency of *Brassica* sp. pollen was found in honey from the airport and considerable amounts of *Allium* sp. in the 18th district.

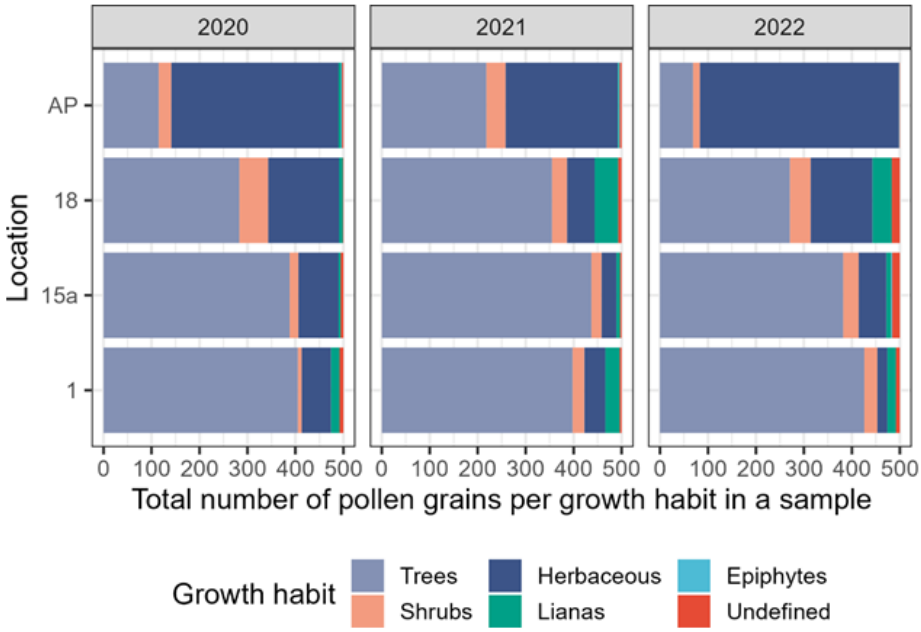

Figure 9. Total amount of pollen per growth habit per 500 counted grains at four selected locations: 1st district Stubenring (1), 15th district Boutiquehotel Stadthalle (15a), 18th district University of Natural Resources and Life Sciences (18), and Vienna International Airport (AP).

3. Discussion

Over a period of three years, pollen diversity in 50 honey samples from 18 different locations in Vienna was analyzed microscopically in order to investigate the foraged plants. Tree taxa were shown to be disproportionally more abundant in honey from urban areas. Potentially, relatively few plant individuals provide mass amounts of nectar and pollen throughout the season.

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3.1. The Role of Trees as Floral Resources

From the top twelve most planted tree species according to the Statistical Yearbook of the City of Vienna [21], seven were also among the most abundant taxa found in the honey samples (Figure 3). They were *Acer*, *Tilia*, *Aesculus* (identified as *A. hippocastanum* and *A.* x *carnea*), *Prunus*, *Pyrus* (identified as *Malus*-Type), *Gleditsia triacanthos*, and *Sophora japonica*. Some of those tree species were planted throughout the 20th century all across town, such as *Acer* sp. and *Tilia* species. *Gleditsia triacanthos* has also been growing along urban and suburban roads for some decades. *Aesculus hippocastanum* has been a popular urban tree for centuries because of its majestic size and inflorescences of ornamental value. *Aesculus* x *carnea* varieties and *Aesculus flava* are planted far less commonly but with increasing tendency [22].

Fraxinus, *Platanus*, and *Robinia pseudoacacia* (Figure 5I,J), which are also frequently planted according to tree records [21], occurred in lesser but still considerable amounts of honey. Other tree species have become popular in the past twenty years, and many individuals have yet to reach a maximum crown size. Those include e.g., *Sophora japonica*, *Liriodendron tulipifera*, and *Koelreuteria paniculata*. *Sophora* was shown to be the predominant pollen type in two samples. Thanks to its high nectar availability, *Sophora japonica* has the potential to be a major floral resource for honeybees wherever it is regularly planted. Pollen from *Liriodendron* and *Koelreuteria* occurred in more than half of all analyzed honey samples, also indicating potential use for the melissopalynological identification of urban origin. *Castanea sativa* is very rarely considered by municipal landscapers yet is sometimes planted by private owners of adequately large gardens.

The availability of nectar-producing trees might positively impact honeybees in particular, as they are polylectic generalists who prefer exploiting mass foraging resources, if available [9,23]. During the flowering season of common tree species in parks and alleys, they find large amounts of food in one place at the same time. Common tree species such as *Tilia* sp. and *Robinia pseudoacacia* are popular sources of monofloral honey in Austria. Their role as mass foraging plants might benefit apiarists as well. Further, the high diversity of trees in cities provides food throughout the season.

In pollen pellets, a decrease in the abundance of tree pollen was observed over the season [24]. In contrast, this study shows that in honey and especially urban honey, the amount may remain stable. The flowers of native trees like *Salix*, *Acer*, *Prunus*, *Rhamnus* (Figure 5E,F), and *Frangula* (Figure 5G,H) are succeeded by *Tilia* and *Ailanthus* in June–July, followed by *Koelreuteria* and *Sophora* in July–August.

3.2. Mass Foraging Plants

In contrast to most wild bees, honeybees prefer the exploitation of mass foraging sources, regardless of whether they are managed or not [25]. As the predominant species in this study underline, these can be agricultural crops or widely planted urban trees of native and non-native origin.

Ailanthus altissima is fast-propagating and tolerant against drought as well as industrial pollutants [26], which makes it well adapted to urban conditions. In this study and perhaps typical for urban apiaries, *Ailanthus altissima* occurred alongside *Tilia* sp., which is also a very common provider of monofloral honey. In the Statistical Yearbook of Vienna [21], *Ailanthus altissima* is mentioned with less than 500 individuals. However, as an invasive neophyte, *Ailanthus altissima* grows in many crevasses and corners in inner city areas, as well as alongside side roads and rail tracks. Those trees likely provide the majority of *Ailanthus* pollen in honey. They are not intentionally planted, yet they are an essential source of nectar and pollen for honeybees and other urban pollinators. This, as well as the mass foraging of "exotic" urban tree species such as *Sophora japonica*, shows the quick process of adaption in honeybees considering new mass foraging resources. The nectar value of *Ailanthus altissima* is 3 (out of 4), with more sugar produced in male flowers [26]. Additional extra-floral nectaries attract pollinators [27]. The main flowering time is June to July. So far, very limited literature on *Ailanthus altissima* as a potential source of monofloral

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honey is available [28]. Honey is said to be of amber color and crystallizes relatively early. The taste has been described as stingingly fruity, resembling the odor of Muscat [29].

Tilia pollen was most abundant in the year 2021, thanks to favorable weather conditions in July at the time of flowering. It peaked in the 1st and 18th districts. Honeybees foraged the numerous *Tilia* trees, which are planted along the Vienna Ring Road, as well as other parks and roadsides. Additionally, in the outskirts of the 18th district, *Tilia* trees occur naturally in the broadleaved forest of the Wienerwald. According to the online tree register of the city of Vienna [22], as many as six distinct species of *Tilia* and four hybrids are intentionally planted within the borders of Vienna.

Besides trees, the most abundant taxa were *Brassica* and *Myosotis*. Each of these was predominant in two honey samples. *Brassica* species are regularly cultivated in the agricultural areas on the north-eastern and south-eastern outskirts of Vienna and were also identified predominantly in honey samples from this area (11th district and airport). *Myosotis* is a common genus of the ground cover. Among the widely identified taxa across all samples were *Plantago* and the climber *Parthenocissus*, which were present in almost all honey samples with a higher abundance in honey harvested later in the year.

3.3. Urban and Suburban Sites

Pollen diversity in honey from Vienna was generally high, which is in accordance with other studies showing the effect of urbanization on the quantity and diversity of pollen in urban honey [30]. Although the flight radius of honeybees in urban and suburban apiaries comprised varying land covers (Figure 8), taxa richness in honey did not significantly differ between the two groups (167 vs. 164 identified taxa, respectively). Mean taxa richness and mean Shannon Diversity Index per sample did not significantly differ either (Figure 1A,B), but taxa composition did (Figure 1C). This was described before by [1] for species richness and composition identified by DNA-metabarcoding of honey.

On the other hand, in this study, the composition of foraged plants differed greatly between samples within the urban and suburban groups, respectively. This was reproducible over the years. Honeybee colonies preferred unique plant taxa even though their flight radius overlapped largely with the 3 km circle around other apiaries. The median estimated flight radius of honeybees in urban areas was shown to be even shorter than in non-urban areas [31,32]. The high heterogeneity of green patches found in cities leads to a more diverse array of foraging plants. Hence, potential food sources are available in even closer proximity to the hive. This might be especially true for mass-foraging plants like trees.

Taxa richness peaked in areas where the flight radius covers both urban and suburban areas and/or stretches beyond the city borders. In the 18th district, the apiary is situated on university grounds in a residential neighborhood next to a large park. The flight radius also covers vineyards and broadleaf forests. There, the number of identified taxa ranged from 44 to 65 over the three years. In the dense urban locations, such as in the 1st and the 15th districts, it was lower. With smaller parks, few private gardens, and fewer recreational areas compared to the suburbs, there are also fewer floral resources available for pollinators. The airport lies outside of the municipal borders but likewise consists of large concrete areas. Within the flight radius are large dry grasslands, agricultural fields, and the riparian forest of the Donau-Auen National Park.

Pollen spectra of specific locations in urban and suburban areas show that the dominance of certain taxa diminishes toward the outskirts. *Ailanthus altissima*, for example, was the most abundant pollen type in the 1st and 15th districts in all three years, with a mean of 53.1% and 32.8%, respectively. In the 18th district, its mean abundance was 12.2%, and at the airport, it was 11.6%. For honeybees, therefore, mass foraging resources might be described as a gradient from the predominance of *Ailanthus altissima* in the concrete inner city towards the predominance of *Brassica* sp. in suburban to rural areas.

3.4. Non-Native Plants May Benefit Foragers

Alien plants are an important food source for bees, especially when they dominate the "floral market" through high relative densities and abundances [17]. Together with the managed native vegetation, they may have a powerful effect on plant–pollinator interactions in urban habitats [3]. For the melissopalynological analysis of honey, however, alien plants might pose specific challenges. In addition to knowledge about the native flora of a region, the identification process requires access to data on what is actually planted in cities.

As the climate is changing and summers become warmer and dryer, municipal and private gardeners have to adapt their planting regimes. They may select plant species and plant compositions that are known to withstand the predicted conditions [19]. Oftentimes, this includes plant taxa that are closely related to native species. One example is *Fraxinus ornus* (Figure 5C,D), which has its natural distribution towards the South and the Eastern Mediterranean. In urban areas, it is slowly replacing the native *Fraxinus excelsior*. While the latter is strictly wind-pollinated, *Fraxinus ornus* depicts a double pollination strategy and is commonly foraged by honeybees, similar to *Castanea sativa* [33]. Further non-native *Fraxinus* species planted in Vienna include *F. angustifolia*, *F. americana*, and *F. pennsylvanica*. The latter is also considered to be an invasive neophyte [34].

Other plant genera have not had representatives in Europe in modern times. *Gleditsia triacanthos* and *Liriodendron tulipifera* are major sources of nectar for honeybees in the Eastern parts of North America and have become common urban trees in cities across Western and Central Europe. Similarly popular are *Koelreuteria paniculata*, *Sophora japonica*, and *Tetradium danielii*, which all originate in Eastern Asia. While the honey value of many native tree species has been described [18,35], information on nectar production and nutritional quality of many non-native ornamental trees has rarely been researched so far [36].

Extensive observations of plant–pollinator interactions have been conducted on urban and ornamental alien plants. However, they usually neglect trees, which, as this study shows, are the major foraging source in inner city environments. Ref. [37] concluded that bee abundance and insect species richness were higher in plots of non-native herbaceous plants. Native plots, on the other hand, would host more specialized pollinators. As [38] discovered, the majority of nectar-producing herbaceous plants and shrubs in urban areas were non-native. Some produced more nectar sugar than native plants, e.g., *Lavandula*, *Nepeta*, and *Mahonia*. Important nectar-producing shrubs were *Berberis*, *Buddleja*, and *Ceanothus* [38]. All of these plants commonly grow in Vienna's green spaces. Single grains of *Berberis* sp. (Figure 7Q,R) and an unidentified 6-aperturate Lamiaceae were present in honey from Vienna, together with pollen from *Buddleja* sp. (Figure 7C,D), which occurred in 29 out of 50 samples (Table 2). Preliminary data from other Central European cities suggest that *Buddleja* might be a major foraging source for honeybees in urban areas nonetheless [39].

3.5. Multiple Factors Influence Taxa Richness

The amount of identified plant taxa varied over the years and did not correlate with the numbers of analyzed honey samples. In 2021, 141 taxa were identified from 20 honey samples at 16 apiary locations, whereas in 2022, 142 taxa were identified from only 11 samples in 11 locations (Table 1). This indicates that taxa richness in urban honey is determined by parameters other than solely location.

One of the most relevant parameters for the foraging behavior of pollinators is the annual weather conditions. Honeybees do not forage in very cold, rainy, or windy conditions [23]. Firstly, foraged flowers only open fully and display their pollen for bees in adequate weather. Secondly, bad conditions impact the honeybees' ability to fly and navigate to the floral source and back to the colony [40]. In 2020 and 2021, the average number of taxa per sample was similar, but taxa composition differed. In the year 2020, the weather in spring was more favorable than in summer, allowing honeybees to forage more spring bloomers, e.g., *Salix* sp., *Acer* sp., and *Prunus* species. In the year 2021, weather con-

ditions were especially unfavorable in springtime but suitable in summer, leading to higher numbers of taxa flowering in June and July. In 2022, there was a considerable increase in taxa richness per sample. Honeybees encountered suitable foraging conditions in spring as well as in summer, leading to the most complete pollen spectra in honey considering the phenological progression.

Another crucial parameter for taxa richness in honey from cities is specific annual planting regimes by private and municipal gardeners. This affects mostly annual and perennial herbaceous species, as well as recently planted trees and shrubs. Oftentimes, planting regimes follow certain recommendations, such as the ability to adapt to urban heat islands [19]. They might also follow the aesthetic sense of planners and landscapers. Similarly, the existence and frequency of unintentional weeds on brownfields and road verges fluctuate over the years. This is due to the high dynamic in planning and construction, in addition to the intensity of chemical and mechanical weed control [2,16,32,41].

Appropriately managed urban green spaces can act as hotspots for pollination services by bees [7]. However, the frequency of mowing and cutting hedges determines the floral availability in tended green spaces. For aesthetic reasons, lawns in inner-city environments are often cut at least once a week, inhibiting herbaceous mass foraging plants such as *Trifolium repens* from developing flower heads. In addition, shrubs such as *Ligustrum vulgare*, *Buxus sempervirens*, and *Ilex aquifolium* are regularly cut into shape, which results in the removal of inflorescences.

3.6. Outlook

Pollen composition in urban honey will likely further change with adaptations of planting regimes to more heat- and drought-resistant taxa. This will likely lead to even more non-native taxa and/or higher abundances of pollen from non-native taxa. Trees hereby have an important synergistic role in urban environments. They require relatively low long-term maintenance compared to herbaceous flower beds, they have a shading effect, and they provide ornamental value to inhabitants of cities. By choosing entomophilous mass-flowering taxa, gardeners can increase and provide foraging opportunities for honeybees throughout future years. For apiarists, the high amounts of non-native trees may benefit honeybee fitness and create new types of monofloral honey in the future. Further studies on this topic should consider the highly dynamic nature of urban beekeeping (e.g., shifts in apiary locations) as well as the specific weather conditions and, potentially, maintenance regimes over the study period.

4. Materials and Methods

4.1. Study Design

For this study, beekeepers were asked to send extracted honey samples from their apiaries within the city of Vienna for three consecutive years. The samples comprised a total of 17 locations in 16 out of 23 municipal districts. In addition, honey from one apiary at the Vienna International Airport was included, which is located approximately 2 km southeast of the municipality border and 20 km from the city center of Vienna. For a complete list of locations and the number of honey samples received per year, see Table 4 and the overview map (Figure 10).

Additionally, four apiary locations were chosen for a more detailed comparison of pollen diversities. These were locations 1 and 15a as representatives of densely built-up urban areas, location 18 as northwestern outskirts within the municipality line, and the airport.

4.2. Materials

All honey samples were extracted by apiarists and received in 240–500 g glass jars. Each sample was from an individual apiary with a minimum of three bee hives. At seven locations, a complete set of samples from three years (2020, 2021, and 2022) was extracted. At the other locations, honey could only be obtained from one or two of these years. This

was due to bad weather conditions limiting honey production or because locations were shifted or abandoned by the apiarists. In the majority of apiaries, honey was harvested one time in the first half of July. At five locations, two harvests were possible (early June and late July to August). All of the delivered jars were either labeled as multifloral honey or they were not labeled at all.

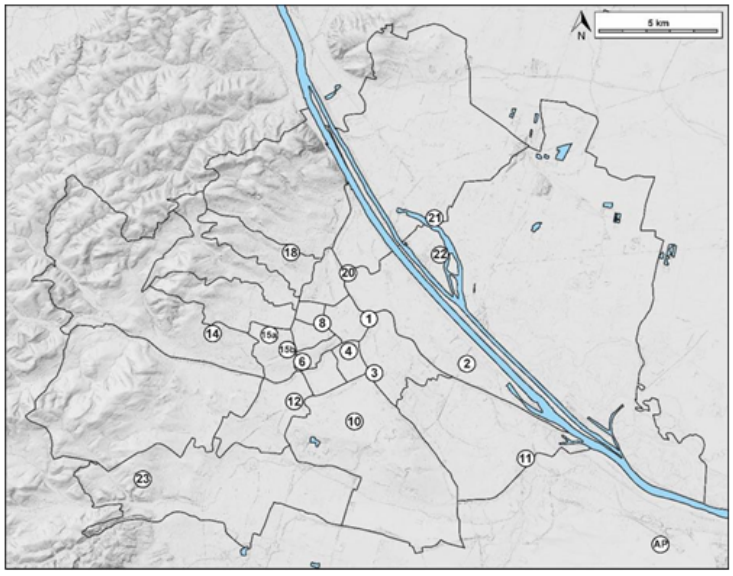

Figure 10. Overview of all 18 locations (numbers according to the respective administrative districts of Vienna, AP = Airport).

4.3. Sample Preparation

All samples were prepared and analyzed according to the harmonized methods of melissopalynology [11,42]. They were thoroughly homogenized before approximately 5–6 g of honey material was dissolved in 10 mL of deionized water in a small beaker on a magnetic stirrer. When fully dissolved, the samples were transferred into a 12 mL pointed glass tube, centrifuged at 3000 rpm (1107× *g*) for three minutes, and decanted. This was repeated a second time to completely finish off the dissolved sample. After the second decanting, the glass tubes were left upside down on a drying rag for approximately 10 min. With a pipette, the sugary deposit on top of the pollen pellet was removed. After 100 µL of deionized water was added, the resuspended pollen pellet was pipetted onto an object slide and dried on a heating plate at 40 °C. Lastly, a drop of unstained glycerol jelly was applied, and the samples were covered with a 20 × 20 mm cover glass and left to rest for at least 24 h.

4.4. Melissopalynological Analysis

Both identification and counting were conducted at 1000-x magnification in oil, using an Olympus BX50 light microscope with an attached digital camera. If necessary, online pollen databases *PalDat* and Pollen-Wiki, as well as other relevant literature, were consulted [43–46]. To examine the relative abundances of the contained pollen types, individual grains were counted out to a total amount of 500 per sample. While pollen of anemophilous species such as Poaceae and Pinaceae were included (as advised by [47]), spores and algae were not. The relative abundances were later assigned to the following categories: predominant (>45%), secondary (15–45%), important minor (3–15%), and minor

pollen types (<3%). In addition, a threshold of 8% was set to determine the overall most abundant taxa in this study.

4.5. Ecology of Foraging Plants

For information on the growth type of most taxa, [48] was consulted. The botanical status of taxa in Austria was retrieved from the List of Neobiota in Austria [34] and integrated into four new individual categories relevant to present and future foraging plants: native/established, invasive, ornamental, and agricultural. Refs. [36,49] provided information on the nectar value of common native and ornamental plant species.

4.6. Land Cover Analysis

QGIS v.3.16.4-Hannover [50] was used to produce an overview map of the 18 locations. The percentual land cover in a 3 km flight radius was calculated based on the "CORINE Land Cover 2018 (raster 100 m), Europe, 6-yearly" dataset [51].

4.7. Statistical Exploitation

Data preparation and analysis were performed with R [52], and graphics were created using the ggplot2 package [53]. The calculation of the Shannon Diversity Index, permutational multivariate analysis of variance, and principal component analysis for taxa composition were carried out using the functions of the vegan package [54].

5. Conclusions

Urban green spaces can provide synergistic benefits for pollinators, apiarists, and city dwellers alike. As a source of nectar and pollen, trees provide mass foraging opportunities for pollinators. This study suggests that the disproportionally high relative abundance of tree pollen from a mixture of European, East Asian, and North American tree species is typical for honey from a Central European city. The most predominant pollen type was *Ailanthus altissima*, which occurred in decreasing abundances from the densely built-up urban center towards the outskirts. General taxa richness did not differ between urban and suburban areas. From a palynological point of view, the increasing popularity of urban beekeeping brings up new challenges for the analysis of urban honey in terms of botanical and geographical origin. The results of this study contribute to the understanding of honeybee foraging patterns in cities and might help decision-makers improve the management of green spaces for pollinators.

Author Contributions: K.K., A.R., J.M., and M.W. conceived this research and participated in the design and interpretation of the data; K.K. performed laboratory work and melissopalynological analyses; J.M.B. performed geographical mapping and produced the graphics; K.W. performed statistical analyses and contributed diagrams; K.K. wrote the paper; K.K., A.R., J.M., M.W., and K.W. participated in the revision of the paper. All authors have read and agreed to the published version of the manuscript.

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5. GENERAL DISCUSSION

5.1. AUTHENTICITY OF URBAN HONEY

Pollen analysis is performed to assess honey authenticity in regard to the botanical and geographical origin of a sample. Urban honey comes with additional challenges. Private and municipal green spaces often hold a great variety of non-native plants, which makes the tracing of geographical origin difficult. “Exotic“ plants may then be accountable for rather unexpected foraging choices of honeybees, which could lead to unintentional labelling errors of botanical origin. But also, the vegetational period itself may be prolonged in cities thanks to the intentional management of urban green spaces.

As the trend towards urban beekeeping is unabated, this research aims at exploring pollen diversity in honey from Central European cities and creating a baseline for future assessments of urban honey. For this purpose, 114 samples from 55 locations in 12 cities were analysed over a period of three years (see Appendix, Chapter I, Figures A1–A5). Large cities with more than one million inhabitants were: Vienna (Austria, 18 locations), Munich (Germany, 11), and Prague (Czech Republic, 1). Medium-sized cities with inhabitant numbers between 200,000 and one million were: Bratislava (Slovakia, 11 locations), Ljubljana (Slovenia, 2), Linz (Austria, 2), and Graz (Austria, 1). Smaller cities with less than 200,000 inhabitants were: Klagenfurt (Austria, 3 locations), Innsbruck (Austria, 2), Salzburg (Austria, 2), Bregenz (Austria, 1), and St. Pölten (Austria, 1). From 114 samples, 82 derived from a single honey harvest between late June and end of August. At some apiaries, two harvests were possible. A first harvest usually occurred from late May to end of June, while the second harvest followed between late July and end of August (16 samples each). For a full sample list see the Appendix, Chapter I.

5.1.1. How diverse is urban honey?

Taxa richness was considerably high with a total mean of 47.1 and a median of 46.5 taxa per sample. The numbers of identified taxa ranged from 15 in the agricultural suburbs of Munich to 73 in the city of Salzburg. The by far highest annual median number was recorded in the year 2022 with 57 taxa. Samples from the second harvest had the highest taxa richness at a median of 49.5, compared to the first

harvest and one single honey extraction. Smaller cities with less than 200,000 contained the highest median number of taxa at 53.

Other studies came to similar patterns regarding the taxa richness of urban honey. Gamrat (2022) observed a greater diversity of foraged plants in urban honey, which favours the production of multifloral honey. This came with the exception of unifloral linden honey when lots of *Tilia* was planted. Richardson (2019) also found a higher diversity and temporal turnover of nectar and pollen foraged in urban compared to rural areas. Lowenstein (2014) relates the higher diversity in urban honey to the foraging range and complex social structures of honeybees, which help them to locate and exploit the scattered floral resources in cities.

Mean Shannon Diversity (H) of samples analysed in this research was at 2.496 with a median value of 2.562, a minimum of 0.704 in the agricultural suburbs of Munich and a maximum of 3.581 in a community garden in Innsbruck. Diversity differed between cities, years, and harvests. Both the minimum and maximum value were recorded in the year 2020. The overall most diverse year was 2022 with a mean value of 2.813. Samples from smaller cities had the highest diversity (2.979), followed by samples from large cities (2.467). Pollen spectra of honey from medium-sized cities were least diverse with a mean of 2.148. The second harvest showed the highest pollen diversity with a mean of 2.743.

Overrepresented pollen types were responsible for the lowest Shannon values and hence the lowest measured pollen diversities. They were *Ailanthus altissima*, *Brassica napus*, and *Castanea sativa*, all of which constituted more than 80% in at least one sample. Some of the samples with the lowest Shannon value had an above average taxa richness, hinting that neither Shannon Diversity nor taxa richness are good indicators on their own and should always be complementing each other.

5.1.2. What impacts pollen spectra of urban honey?

A multitude of parameters influence the foraging behaviour of pollinators in urban areas. On a landscape scale, impervious surfaces favour larger and fitter insects like the honeybee, which can navigate to farther away floral resources than smaller wild bees (Lowenstein et al. 2014; Baldock 2020). On a more local scale, it is the

access to diverse urban green spaces with an anthropogenically enriched floral abundance.

5.1.2.1. Landscape parameters

Biodiversity at the landscape level typically combines the alpha diversities measured at multiple local sites into regional models. Key parameters determining the nature of native vegetation in a city are its altitude and latitude. They impact, e.g., the air temperatures, determining flowering season and hence also the length of the foraging period for honeybees (Rajagopalan et al. 2024). The particular topography of a city may also define drainage basins and wind intensities, impacting growing conditions for plants. Apart from these variations, the analysed apiaries were all located in urban areas in the same bio-region with temperate climate, temperate deciduous vegetation, and four distinct seasons (Ellenberg et al. 2010).

Urbanisation is said to selectively promote generalist plants and animals which are able to adapt and thrive in urban climates. This is referred to by the term “urban biotic homogenisation” (UBH, see McKinney 2006; Lokatis and Jeschke 2022). This was observed especially for urban pollinator communities (Deguines et al. 2016; Remmers and Frantzeskaki 2024; Sinno et al. 2025). On the contrary, several studies in the recent past have pointed out that through active management and integration of horticultural elements into urban green spaces, the UBH effect may be overruled by heterogeneity on the local level (Neumann et al. 2024; Sexton et al. 2025). The pollen spectra identified in this research do not support a general homogenisation theory for urban foraging plants.

5.1.2.2. Local parameters

On a local scale, the heterogeneity of urban green spaces supports a wide array of foraging plants (Philpott et al. 2020; Zaninotto et al. 2023; Neumann et al. 2024; Sexton et al. 2025). Among the drivers of local plant diversity are parks, private gardens, allotments, road verges, cemeteries, industrial sites, seminatural vegetation, urban farms, vineyards, inner-city rooftops, and balconies. According to Lepczyk (2017), the total size of urban green spaces is secondary to the quality of green spaces, which describes a continuum from intact remnant vegetation to highly altered cultivated patches. The selection of plants which are intentionally grown is

largely influenced by socio-cultural and economic factors (Lowenstein et al. 2014; Philpott et al. 2020). In this research, for example, multiple identified taxa could be correlated with nearby communal urban gardening patches. They were *Capsicum* sp., *Citrullus lanatus*, *Cucurbita pepo*, *Ocimum basilicum,* and *Solanum lycopersicum*.

In private spaces, the identity of floral resources depends, e.g., on the experience, motivation, education, gender, and ethnical background of gardeners in addition to the underlying environmental conditions (Philpott et al. 2020). This applies to both private and urban plant arrangements. As honeybees prefer mass foraging resources like urban trees in parks and along roadsides (see Chapter 4.C.), municipal planting regimes may be much more vital for honeybee nutrition in the inner city than in the suburbs where private gardens and ruderal vegetation are more common. Municipal planting lists incorporate a highly diverse set of plant species and seem to follow common recommendation in all included cities. This could be confirmed by the presence of “exotic“ urban trees such as *Koelreuteria paniculata*, *Gleditsia triacanthos*, *Sophora japonica*, and *Liriodendron tulipifera* in the pollen spectra of urban honey.

On the other hand, several parameters negatively impact honeybee foraging in urban areas on a local scale. Honeybee learning was shown to be negatively affected by traffic induced air pollution, as petrol exhaust disguises the volatile organic compounds used by honeybees to detect nectar and pollen traces (Leonard et al. 2019). In the present research, none of the participating apiarists kept their honeybees directly adjacent to heavy traffic. There was at least one line of houses and trees in between, requiring the bees to navigate upwards, away from the traffic on the ground. Whether the honeybees missed out on specific foraging opportunities due to car traffic (e.g., on herbaceous plants in road side verges) is not known. The pollen types identified here did not point to a relevant influence of noise pollution or rooftop distance to the ground either.

5.1.2.3. City size and urbanisation gradient

Median taxa richness and median Shannon Diversity were the highest in samples from small cities with less than 200,000 inhabitants. Median taxa richness was almost identical in large cities (> 1 million) and medium-sized cities (200,000 –

1 million). Median Shannon Diversity was higher in larger cities than medium-sized cities. Reasons for such variations may be overlapping ecotones in small cities and suburban areas. Garden centres sell the same array of ornamental plants anywhere, enhancing diversities in municipalities of all sizes. However, in small cities, the flight radius of urban honeybees is more likely to include higher proportions of the surrounding semi-natural and agricultural vegetation, as well as agricultural weeds spreading into built-up areas. The suburban belt of larger cities may mimic these conditions with flight ranges covering ecological ecotones. In the centre of larger cities, composition changed compared to suburban sites, and seminatural elements were replaced by a higher proportion of ornamental and/or neophytic plants (see Chapter 4.C.). Taxa richness, on the other hand, did not differ. This confirms what, for example, Noël (2023) reported for the urbanisation gradient in Tokyo. An increase of non-native plants towards the city centre was also reported by Fox (2022) and Richardson (2019).

In terms of topography, all cities in this research have a river or larger body of water that hosts riverine vegetation along its banks. Small cities like Innsbruck and Salzburg, which showed some of the highest pollen diversities in honey, are additionally located in mountainous regions with access to multiple altitudinal zones and pastures. They included pollen taxa such as a Ranunculaceae (up to 33.6% in Innsbruck), and an Ericaceae type, possibly related to the widely occurring *Vaccinium myrtillus* in the surrounding spruce forests. The city of St. Pölten lies within an agricultural area but contains a high proportion of managed green spaces. Non-native urban trees played an equally important role in honeybee foraging there than in larger cities. Pollen spectra from medium-sized cities like Ljubljana and Graz showed some of the lowest Shannon Diversity values. This is possibly due to their location in a slightly warmer climatic region combined with the natural distribution of *Castanea sativa* in the native vegetation. The high relative abundances of *Castanea sativa* pollen (up to 85.2% in Ljubljana and up to 75.6% in Graz) are responsible for a bias.

5.1.2.4. Foraging “exotics“

Taxa richness and diversity in urban honey analysed for this research have been boosted by the presence of pollen types from non-native ornamental species (see

Chapter 4.C., Table 3). Indeed, non-native species are responsible for a high local plant diversity in urban areas (Lepczyk et al. 2017; Noël et al. 2023). The repertoire of municipal green spaces has grown over time and reflects past and current trends. For example, the now invasive *Ailanthus altissima* has been imported to Central Europe as a decorative boulevard tree in the 18th century (Punz et al. 2004). While pollen of plants belonging to the native deciduous vegetation could be identified in all samples, the proportion of non-native taxa varied between apiary locations, harvesting seasons, and years.

Zaninotto (2023) observed that non-native plant species were more often visited by generalist foragers such as honeybees. These plants are nonetheless an important component of local plant-pollinator networks in summer when native floral resources are scarce (Sponsler et al. 2020; Noël et al. 2023; Zaninotto et al. 2023). Today, aesthetic reasons and changed climate-adaptive requirements are both considered when introducing new non-native plants to urban green spaces (Sjöman et al. 2012; Stadtsenat Graz 2023). Several species have been introduced to Central Europe as urban trees relatively shortly ago and their impact on local plant-pollinator interaction could not be studied yet. They include, e.g., *Nyssa sylvatica*, *Cladrastis lutea*, *Parrotia persica*, *Phellodendron amurense*, and *Asimina triloba*. They were not identified in this research of urban honey, but might present themselves in future pollen analyses.

The origin of herbaceous horticultural plants as native or non-native seems to be less important than functional traits of flowers, e.g., sterile hybrid varieties or cultivars with double corolla flowers (Garbuzov and Ratnieks 2014). For example, the non-native *Salvia splendens* is planted in a lot of European cities (BMLRT 2021). It is, however, inaccessible to honeybees due to the long corolla, which is a results of coevolution with hummingbirds (Corbet et al. 2001).

5.1.2.5. Weather conditions

Different annual weather conditions were responsible for a great part of variation in the pollen diversity of honey samples from 2020, 2021, and 2022. For example, in the city of Innsbruck, honey was foraged mostly from spring-flowering plants in 2020 and mostly from summer-flowering plants in 2021. This can be explained by the timely onset of flowering in April–May of 2020, followed by a period of unstable

weather conditions in the summer. In 2021, spring foraging was inhibited by a later rise in temperatures (GeoSphere Austria 2025) and a delayed development of flowers. Frosty nights during the blooming of *Prunus*, *Malus*, etc. have caused irreversible damage to the flowers (MeinBezirk Tirol 2021). Furthermore, hail storms damaged the flowers of fruit trees and crops in the summer months (GeoSphere Austria 2025). In agricultural areas, many fruit farmers protect their crops from frost and hail damage by installing heating systems or draped hail netting. Ornamental trees, which are important foraging sources for pollinators (see Chapter 4.C.), are usually not protected and may suffer more damage to their flowers by extreme weather events. The foraging season 2022 was marked by stable weather conditions with warm temperatures early on and sufficient precipitation in May–June (GeoSphere Austria 2025). This led to an accelerated development of plants and supported foraging throughout the flowering season. As a result, the highest median taxa richness in honey was found in 2022, where spring-flowering and summer-flowering taxa were equally present.

5.1.2.6. Climate change

Drought has a negative effect on nectar production of plants through a decrease of plant height, floral units, corolla size, and nectar-sugar concentrations (Quinanzoni et al. 2024). While climate change induced drought is a fairly new challenge for the natural vegetation in Central Europe on a landscape level, urban green spaces are already experiencing drought on a local level (Oke 2017). As such, planting choices in cities must consider resilient species that are able to withstand present and future climatic conditions. Sjöman (2012) describes the search for climate change adapted urban trees in different natural regions. Lists of recommended species are available (Stadtsenat Graz 2023; Stadt Wien 2025b). They comprise ideas that mimic the naturally adapted vegetation of the Mediterranean region and continental steppes (BMLRT 2021). In addition, adapted plant assemblages should provide a variety of functional floral traits to support all pollinator groups (Quinanzoni et al. 2024).

Examples of recommended climate change adapted species found in urban honey samples are *Fraxinus ornus*, *Gleditsia triacanthos*, *Koelreuteria paniculata*, *Platanus* sp., *Pterocarya fraxinifolia*, and *Sophora japonica* (Stadtsenat Graz 2023). Herbaceous species recommended for drought conditions and detected in the urban

honey samples include species of the genera *Achillea*, *Allium, Salvia*, and *Sedum*. Shrubs included *Buddleja* sp. and *Helianthemum* sp. (e.g., Grüner Daumen 2024).

Another serious climate-change-related issue for honeybee foraging is the elongation of the foraging season by warmer temperatures in early spring and late autumn. According to Rajagopalan (2024), warmer temperature could be linked to a decrease of honeybee overwintering success. They are able to alter the age structure of honeybee colonies. Autumn foragers die earlier, which requires younger bees to start foraging prematurely and with a lack of experience, leading to decrease in foraging success. In the urban climate, this problem may be enhanced by the intentional planting of ornamental species which flower in early spring or into late autumn. Whether this could have implications for the viability of urban beekeeping is not known. However, foraged plants with flowering times past the annual honey extraction date(s), are usually not registered in melissopalynological honey analyses. Here, the identification of corbicular pollen pellets collected later in the year may lead to a better understanding (see Lau et al. 2019; Brodschneider et al. 2019; Noël et al. 2023).

5.1.2.7. The effect of green space maintenance

The impact of green space management is noticeable in European city centres. They include lawn mowing, trimming of hedges, and the removal of unwanted weeds. In parks of the inner-city centre of Vienna, which are maintained to a high degree and serve aesthetic services for locals and tourists alike, the mowing frequency of lawns is generally higher. Elevated mowing frequencies are responsible for decrease in the availability of herbaceous flowers such as *Trifolium repens*, which is otherwise a mass foraging resource for honeybees (Lowenstein and Minor 2016; Kanduth et al. 2021; Noël et al. 2023). The effect of manicuring could be confirmed by the low amounts of *Trifolium repens* pollen in urban honey from this research. Despite having been present in almost all honey samples, the average percentage of *Trifolium repens* pollen across all analysed samples was 2.2 with a median of 1. It surpassed the 15% threshold in only one sample from the city of Salzburg.

The contribution of spontaneous weeds on pavements and built structures for urban biodiversity has often been neglected (Bonthoux et al. 2019). Not surprisingly, areas

with recent asphalt pavement support hardly any plant life compared to cracked asphalt and sandy pavements. The following taxa, attributed to spontaneous herbaceous vegetation, were identified in urban honey: Species of the genera *Achillea*, *Artemisia*, *Convolvulus*, *Geranium*, *Geum*, *Lotus*, *Myosotis*, *Papaver*, *Plantago*, *Potentilla*, *Reseda*, *Rumex*, *Sedum*, *Taraxacum*, and *Veronica*, as well as various other Asteraceae, Brassicaceae, and Chenopodiaceae species. Identified woody plants associated by Bonthoux (2019) with pavements include *Ailanthus altissima*, as well as *Berberis*, *Buddleja*, *Hedera*, *Parthenocissus*, and *Syringa* species. Urban planners are advised to use an intersectional approach to deal with spontaneous pavement vegetation, which conserves resources for pollinators while not neglecting issues like disability-access.

When it comes to urban trees, gardeners have the choice between a wide range of entomophilous and anemophilous tree species. Wind pollinated trees such as *Celtis australis*, *Celtis occidentalis*, *Corylus colurna*, *Ostrya carpinifolia*, *Pterocarya fraxinifolia*, *Zelkova serrata*, as well as various *Cedrus*, *Pinus*, *Populus*, *Quercus*, and *Ulmus* species are recommended for climate-adaptive urban planting (Stadtsenat Graz 2023). They typically contribute little to pollinator nutrition and their isolated presence in the pollen spectra from urban honey may rather have been through contamination. *Quercus* pollen, with up to 4.4 percent, is an exception which may be attributed to the foraging of honeydew on oak trees (Heimbach 1986).

Furthermore, the anemophilous *Chamaecyparis lawsoniana*, *Taxus baccata*, and *Thuja occidentalis* are often planted for privacy measures. Alternatively, dense hedges can be grown from *Acer*, *Berberis*, *Cotoneaster*, *Crataegus*, *Ilex*, *Ligustrum*, and *Photinia* species, which provide foraging opportunities for honeybees. Planting choices must be carefully considered with regard to their impact on urban dwellers and their health. This includes, for example, the allergenic potential of wind-pollinated species, and the potential of bee stings in close proximity to mass foraging resources during flowering time.

5.1.3. Beekeeping in Central European cities

5.1.3.1. What's different compared to rural areas?

Urban beekeeping differs from beekeeping in rural areas in various environmental, ecological, and socio-economical parameters. The foraging distance of honeybees in urban apiaries is reportedly shorter (De Vere et al. 2017) and can be as low as 688 m on average (Dietz 2018). Whether this applies solely to pollen foraging (Fox et al. 2022) or also nectar foraging is not fully understood yet. Urban climatic conditions on the macro- and microscale give rise to an altered assemblage of flowering plants. These foraging resources alter the weight gain pattern in honeybee colonies, too, which was determined by weighing the hives (Komasilova et al. 2023). Lastly, also frequent shifts in apiary location can lead to highly dynamic changes in pollen spectra of urban honey.

5.1.3.2. When to harvest in cities?

Honey yield as well as the frequency and time of honey harvesting in cities is influenced by the urban climatic conditions on the macro- and microscale and may differ from the surrounding countryside. Urban drought conditions are to a high degree counterbalanced by maintaining efforts for green spaces, which often include daily irrigation regimes throughout the vegetation period. In this way, urban green space management leads to a different pattern of honey accumulation. While rural apiaries usually show a sharp peak from the blossoms of agricultural crops in May-June (e.g., through rapeseed *Brassica napus*), the accumulation of honey in urban hives follows a more linear increase with moderate but equal gains throughout the season (Komasilova et al. 2023). Extraction and yield of urban honey is therefore also impacted by weather conditions in another way: A rainy spring might not be critical yet. However, a rainy summer in addition to a slower honey accumulation in spring may lead to a failed harvest, from a beekeeper's perspective. It may even put the colony's survival at risk (Rajagopalan et al. 2024).

Whether honey is extracted once, twice, or even three times, also largely depends on the personal choice of the beekeeper and/or their range of honey products (Crane 1990). In order to sell unifloral honey from urban areas, plant phenology needs to be closely observed. A first harvest may then take place between the flowering times of *Robinia pseudoacacia* and *Tilia* species in May–June, which

allows the build-up of unifloral *Tilia* honey as soon as the nectar-rich linden trees start to flower (June–July). The second harvest may be squeezed in before the start of the *Ailanthus altissima* flower in the second half of July, in order not to spoil the distinct taste of *Tilia* honey with the strong and spicy taste of *Ailanthus* nectar (Gardi 2020).

Lastly, the total yield is also determined by the topography of the city, the latitude, altitude, and whether the flight radius around the apiary covers a large body of water like a lake or an ocean. In Vienna, a hive might produce up to 38 kg or more of honey annually (EVVA Sicherheitstechnologie 2022). In the city of Bregenz, the beekeeper reported an average honey yield of only 4 kg per hive in 2020 and was not able to extract honey in 2021 at all. This may be attributed to the location of the city, which is located between Lake Constance and the Pfänder mountain, as well as specific climatic conditions that support little agriculture.

5.1.3.3. Site dynamics

Urban honey samples were collected directly from beekeepers after they confirmed that the apiaries would be available for the duration of the research from 2020–2022. However, it became clear in the second year that sites would shift or drop out due to various reasons. Because of its rising popularity, lots of apiary sites are being newly established every year. They include private gardens, corporate grounds, as well as cooperations with landmarks and institutions (HYPO NOE 2019; Hotel Daniel Vienna 2025; Mestna občina Ljubljana 2025). Sites may be short-lived though. Beekeepers might not be content with the quality of honey that they harvested from a specific location and hence seek urban sites with a better availability of foraging plants for the oncoming season. Others will gladly move their hives to a more famous location or landmark when possible. Additional challenges may arise which are peculiar for urban areas: Plots of remnant vegetation might be built on or developed into other urban features, legal challenges might mean that beekeepers lose the permission to access a certain property, or cooperations come to a halt when the novelty wears of. Such dynamics are to be acknowledged in addition to other parameters which determine the diversity of pollen spectra in urban honey and its geographical authenticity.

5.1.4. Implications for the labelling of urban honey

5.1.4.1. Multifloral honey

This research shows that pollen diversity of urban honey is comprised of a diverse mix of native and non-native taxa from various origins, notably elements of the South-East European, North American, and East Asian flora. They usually occur together in one honey sample. As such, urban honey is to be strictly distinguished from mixtures of honey from different countries as in the vague labelling term “blend of EU and non-EU honeys” (EC 2002), which refers to multiple geographical origins. This specific composition of urban honey should be taken into account for new guidelines regarding the origin and authenticity of food products, which are currently under development (EC 2024).

5.1.4.2. Unifloral honey

Thanks to the altered foraging sources in contrast to rural areas, a specific set of unifloral honeys can be yielded in urban areas. Cities in Central Europe have a long tradition of planting native and non-native *Tilia* species (Milecka et al. 2019; Dmitruk et al. 2024), therefore linden honey has historically been one of the most commonly produced unifloral urban honey (Von der Ohe et al. 2016). In this research, lots of honey samples from Munich and Vienna fulfilled the melissopalynological criteria of unifloral *Tilia* honey with up to 57% relative abundance. However, while samples from Munich presented with the distinct taste and smell of *Tilia* honey, the samples from Vienna were strongly dominated by the olfactory characteristics of the co-occurring *Ailanthus altissima*.

So far, descriptions of unifloral *Ailanthus* honey are insufficient. In the list of botanical species giving unifloral honey in Europe (Persano Oddo et al. 2004), the occurrence of *Ailanthus* honey is given as ‘rare’ and limited to Italy, Spain, Germany, and France. For urban honey, an update to ‘medium’ or even ‘abundant’ should be considered. Alternatively, and due to the similar plant composition in cities across different countries, a separate section for main components of ‘urban’ honey may be helpful. The distinct features of the respective honey samples further point out the need for refined olfactory and melissopalynological criteria and the establishment of a physico-chemical reference profile for *Ailanthus* honey (with or without co-occurring *Tilia*).

Also, the addition of *Sophora japonica* to the list on species giving unifloral honey (Persano Oddo et al. 2004) must be considered, as it was the most abundant pollen type in some honey samples, with up to 63%. "*Sophora*" honey can be found online from sources in the Black Sea region and in China (VDAO 2025), yet, criteria on the authenticity analysis of *Sophora* honey are absent from literature. Because of the late bloom of *Sophora japonica*, such honey cannot be extracted before mid-August, which is rather late in the season.

5.1.4.3. How specific is too specific?

In general, the labelling on the jar must adhere to harmonised traceability requirements for the geographical origin of honey (EC 2002, 2024), which must be confirmed by melissopalynological analysis. As such, a distinct label is permitted only when the honey was foraged fully or mainly from a distinct vegetation type that is exclusively found in the region of the apiary (EC 2002). In this research, a very similar set of ornamental urban plant species was found in cities across Austria and the neighbouring countries. It is therefore not clear how to distinguish between urban honey, e.g., from Vienna and Bratislava, or Graz and Ljubljana, although the historical openness to integrate non-native park trees might still show some subtle city-specific differences.

It is further a common trend to label urban honey with the locality of a specific apiary. They are most typically a tourism landmark or the rooftop of a certain building. It may also state the neighbourhood or district in which the honeybees were kept. While such labels surely boost the marketing of the respective honey, it remains legally required to additionally state the country of origin (EC 2024).

5.2. ESTABLISHING A REFERENCE BASELINE

Correct and plausible identification of pollen grains is the basis for any pollen analysis and melissopalynological assessment. However, due to convergent evolution in different plant taxa and morphological similarities between related species, the process of identification is not always so straight-forward. Reference databases and method diversification are essential and can lead to improved results.

5.2.1. Pollen identification with databases

Pollen databases usually work through optic comparison of micrographs and assist to find the most plausible taxon of origin. Advantages of such platforms are the ability to store collected images, keep information on the characteristics of each pollen type, and provide the opportunity to share and discuss pollen grains with a larger community of palynologists. Over the last decades, the quality of published reference material has improved thanks to the rapid advancements in digital imaging.

5.2.1.1. Accessibility and scope

Free accessibility of references improves the overall quality of applied palynological studies. *PalDat* (PalDat 2025, see Chapter 4.A.) is a non-profit online pollen database that does not require a sign-in, any form of payment or membership in an institution in order to access pictures and information. By welcoming users to submit their own data, *PalDat* continuously increases its number of datasets. From its beginnings, *PalDat* was programmed to be inclusive of all microscopical methods such as LM of hydrated and acetolysed pollen grains, SEM of hydrated and acetolysed pollen grains, as well as TEM. This approach helps to increase the range of portrayed plant species without limiting the availability of reference material. In order to guarantee high standards of published pictures and data, every contribution on *PalDat* is peer-reviewed by expert palynologists. The availability of references with a worldwide scope is essential for identification of pollen types in all sorts of applications. Melissopalynological studies on the geographical origin of honey benefit greatly from open access reference databases, which contribute to the identification of “exotic” pollen grains in local honey samples (see Chapter 4.C.), as well as in detecting fraud in globally traded bulks (e.g., Soares et al. 2017). Besides its scientific purpose, *PalDat* aims at triggering curiosity among a non-academic audience, which includes beekeepers and honey consumers (Weber and Ulrich 2017).

The establishment of a functional online pollen database requires several key ingredients: adequate server infrastructure, programming skills, practical palynological advice, and full funding. Successful cooperation and communication between programmers and scientists are vital to develop a good understanding for

each other's work. For the integration of the former pollen database PONET into *PalDat* (Chapter 4.A.), multiple meetings were held, responsibilities were discussed, specifications were written down, a test version was created, and the legal situation of ownership and copyright was handled. Parallel to this, financial resources were secured and roles were allocated in order to guarantee the long-term operation of *PalDat* after the initial fusion. Unlike other databases, *PalDat* is not run by an academic department, institutional organisation, or individual persons, but by the "AutPal - Society for the Promotion of Palynological Research in Austria" (AutPal 2025). Being a non-profit association of members with ties to academic research and applied use, AutPal can operate the *PalDat* database independently and keep the open access, without relying on institutional politics.

5.2.1.2. Other melissopalynological references

Multiple other helpful reference databases are available besides from *PalDat*. However, not all can be accessed by the broader palynological community. Limits to the accessibility of online databases may be copyright issues, lack of sustainable funding, lack of IT-management and secure server structure, competing mentality among institutions, their establishment for a one-time project, or their dependence on one person who might retire or move away. As of 2024, several other pollen databases were freely accessibly online. They were, e.g., Pollen Wiki (Stebler 2025) for hydrated pollen and the Australasian Pollen and Spore Atlas (Haberle et al. 2021) for acetolysed pollen. Pollen Wiki contains pictures of stained pollen reference samples which were produced especially for this purpose. Other databases such as the PollenFlora of Italy (Accorsi 2010) include mainly images from older literature. Pollen from study sites in North America is available on Discover Life (2025). Common issues with online databases are outdatedness, poor traceability of sources, and confusing layouts.

Over the past century, numerous printed books with collections of pollen micrographs have been published. They include different microscopical methods (LM acetolysed, LM hydrated, and SEM) and may have a geographical or taxonomical focus. When adequately pictured and arranged, such printed material can be invaluable for the identification of pollen types.

5.2.1.3. Molecular databases for DNA-metabarcoding

Apart from microscopic methods, DNA-metabarcoding is the most widely used pollen identification method in research today (Soares et al. 2023). In order to correctly identify the origins of the material, precise molecular references are needed. Laboratories usually build their own data analysis pipeline to process DNA-based results (De Vere et al. 2017; Milla et al. 2021). This involves the creation of a customised database. Most commonly, reference sequences for foraging plants are downloaded from large international nucleotide databases such as NCBI (Sayers et al. 2022) or the EMBL Nucleotide Sequence Database (Stoesser et al. 2002). Other reference collections focus on the specific primer system, such as ITS2 (Banchi et al. 2020) and *rbcL* (Bell 2021). Additionally, local nucleotide databases may be available which include the most widespread taxa in the respective geographical area (Bänsch et al. 2020). The sequence reads, which are obtained through DNA metabarcoding, are then trimmed and merged before being matched against the customised database using megablast (Smart et al. 2017; Milla et al. 2021; Fox et al. 2022). When sequence reads from honey are absent from the previously constructed customised database, they may be added manually after a blast search on NCBI (Sponsler et al. 2020). This might especially apply to ornamental, agricultural, or newly invasive plants.

Just like with microscopic methods, sufficient floristic knowledge is mandatory when processing the results. Plausibility can become an issue when botanical characteristics are neglected. They might concern the plant's biogeographical distribution, pollen distribution mode, the flower's morphological accessibility to pollinators, and flowering time. A common error occurs during taxonomic identification, when, for example, closely related taxa have nearly identical nucleotide sequences (Richardson et al. 2020). Blast search might find a 100 % correlation with sequence references from closely related plant species. This might especially be the case for the *rbcL* and *trnL* primer systems, which are very conservative and hence might elude potential differences between closely related plant species (Soares et al. 2023). Other studies processed their results by setting quantitative thresholds for sequence reads and manually combining reads of closely related species. The latter may reduce the taxonomic resolution down to genus or

family level, but the authenticity of results increases (Smart et al. 2017; Bänsch et al. 2020; Fox et al. 2022).

5.2.2. Producing reference samples

Databases and printed literature contain information retrieved from pollen samples, which were purposely prepared to serve as references. All plant species must be correctly identified botanically before being sampled. During anthesis of the flowers, several fresh anthers are collected in fresh paper bags. It is advised to herbarise the plant and/or take pictures of habitus and flowers for documentation (Halbritter et al. 2018). Habitus pictures of plants may be added to datasets as well. The botanical content of urban honey is often unclear as many “exotic” species are planted intentionally in urban green areas such as parks and gardens (Harrison and Winfree 2015; Fox et al. 2022). Availability and access to a wide array of references with a global distribution is invaluable for pollen identification. Reference slides produced for this PhD research focus on plant species relevant for the identification of urban honey, which were not previously published on *PalDat* (see “List of Publications”).

5.2.2.1. Light microscopy

A picture plate describing the preparation of hydrated material for light microscopic reference slides can be found in Appendix (Figure A10). To depict method diversity, all pollen samples were embedded in both unstained and stained glycerol gelatine. Basic fuchsin was used for staining (Mulisch and Welsch 2015). During the analyses of hydrated honey samples, pollen types were compared to reference slides of urban and horticulture plants which were sampled specifically for this research, in addition to consulting *PalDat* (PalDat 2025) and Pollen Wiki (Stebler 2025). Additionally, fresh anthers of selected plant species were acetolysed to produce reference slides for the acetolysis method. These purpose-made LM reference were helpful in the identification of *Zingiber* pollen in a sample from Munich (see Appendix, Figure A11) and pollen of *Gleditsia triacanthos* in various acetolysed samples of Chapter 4.B. (see Appendix, Figure A12).

5.2.2.2. Electron microscopy

Electron microscopy is rarely used in routine melissopalynological analyses. Nonetheless, it can be helpful during the process of pollen identification when

particular characteristics distinguishing taxa can only be observed in the scanning electron microscope (SEM) or the transmission electron microscope (TEM). The preparation of samples is more tedious than for LM and requires a well-equipped laboratory with a fume hood. For this research, SEM references of acetolysed pollen from selected urban plant species were prepared (e.g., *Gleditsia triacanthos*). For that purpose, the freshly collected anthers were air-dried and acetolysed before investigation. Alternatively, Critical Point Drying (CPD) can be used to depict the size and shape of hydrated pollen grains in SEM (Halbritter 1998).

5.2.2.3. Quality and editing of reference data

Reference material needs to be somewhat cohesive in its appearance in order to compare pollen characteristics and micrographs to each other. For imaging, a certain resolution and magnification is required in order to examine, e.g., pollen wall ornamentation and aperture configurations (Halbritter et al. 2018). Further, the correct scale bar should always be inserted into the picture. For the benefit of highlighting the pollen grain and its morphological attributes, the image may later be given a monochrome background. Brightness and contrast may be adjusted. The pictured pollen grain itself must never be altered by image editing software so not to distort relevant pollen characteristics. For *PalDat* (PalDat 2025), any new submission should follow the guidelines under “Instructions for Authors” on the website.

5.3. COMPARING THE RESULTS OF STANDARDISED METHODS

Honey can be called authentic when its production and ingredients are in accordance with the legal requirements, when it is distributed under the correct label and does not claim to be of any other botanical and geographical origin than what it actually contains (EC 2002; Von der Ohe et al. 2004; Soares et al. 2017). The specific method in use must never alter the conclusion of the analysis in regards to authenticity. Any method of melissopalynology must therefore be previously validated in test runs. At a minimum, a method should be able to identify the most relevant pollen types qualitatively and quantitatively, before being put into applied use.

5.3.1. Light microscopic methods

Typically, laboratories use either the hydrated or the acetolysed method of melissopalynology in LM for analysing unknown honey samples. In Chapter 4.B., a careful comparison between the two methods was conducted. The hydrated method allows to examine characteristics such as the atrium (Betulaceae, Cannabaceae, Moraceae) or cytoplast infoldings at the aperture (Rosaceae). Characteristics of the pollen wall and the aperture margins increase in visibility during acetolysis. The picture plates in Chapter 4.B. show side-by-side, how pollen of the same plant taxon may look different when applying the different LM methods.

5.3.1.1. Qualitative analysis with LM

In hydrated samples, pollen grains appeared swollen with a rounder shape and no condition-based infoldings. The pollen wall surface was sometimes obscured by pollenkitt (Halbritter et al. 2018), yeasts, cytoplast contents of destroyed pollen grains, or other organic debris. Yet, it was possible to identify and count such partially obscured pollen grains through other morphological characteristics. Overall, identification success with the hydrated and acetolysed method was comparable when it comes to the most relevant pollen types. The largest divergence was in the category of minor pollen (< 3%). They often occur as single pollen grains in a sample and their presence on the prepared slide might be coincidental. However, the total number of identified taxa was considerably higher with the hydrated method. A larger amount of pollen grains on the slide makes the coincidental occurrence of minor pollen types more likely. Studies on pollen diversity, bee nutrition, and environmental monitoring should therefore consider the use of the hydrated method, if possible.

5.3.1.2. Relative quantitative analysis with LM

For quantitative estimations, pollen was identified and counted out according to taxa. In the comparison study (Chapter 4.B.), the relative abundances in the hydrated and acetolysed methods were very similar when it comes to the most relevant pollen types, proving that the two methods may be interchangeably used for routine analysis. While the precision is relatively weak at lower frequencies, it stabilises at around 500 pollen grains and only slightly increases when counting to 1000 (Von der Ohe et al. 2004; Weng and Hooghiemstra 2024). During the analyses

of the 2020 honey samples with the hydrated method, counting continued to 1000 in three samples. At the “Salzburg Hotel Auersperg” site, the number of identified taxa increased from 45 (500 counted grains) to 49. At the “Innsbruck Kapuzinerkloster” site, the taxa number increased from 66 to 68 and at the “Innsbruck Paschbergweg” site, it increased from 62 to 68. None of the additional taxa had a relative abundance of > 1%. For studies on the total diversity of foraged pollen types, such as biomonitoring studies with honeybees, counting to 1000 might be beneficial. For melissopalynological routine analysis, 500 pollen grains is sufficient.

The presence of very dominant taxa on the overall representation of diversity in the results may be mitigated by counting all “others” to a statistically relevant number (Weber et al. 2020). In 2021, this was experimentally conducted for three honey samples, where all “others” were counted to 500. At the “Graz Brückenkopfgasse” sample, the percentage of predominant *Castanea sativa* pollen increased from 75.60% to 81.82% at 2750 counted pollen grains, while the taxa number remained the same at 40. At the “Ljubljana SKB21” sample, the percentage of *Castanea sativa* pollen increased from 69.80% to 76.47%, at 2125 counted pollen grains, while the taxa number increased from 52 to 55. At the “Ljubljana P4421” sample, the percentage of *Castanea sativa* pollen increased from 79.80% to 84.97%, at 3327 counted pollen grains, while the taxa number increased from 50 to 57. The increase of very small predominant pollen types such as *Castanea sativa* suggests that counting the “others” to 500 may be the most precise method, especially when the screening and identification of all pollen types on the slide (as in Von der Ohe et al. 2004 and in Chapter 4.B.) is the base of counting lists. In applied melissopalynology, the mode of counting pollen grains might impact the declaration of unifloral sweet chestnut honey, which is legally required to contain at least 90% of *Castanea* pollen (Beckh and Camps 2009; DLMBK 2023) — with a tolerated minimum of 86% according to Von der Ohe (2004) when the other physico-chemical parameters are in line with unifloral *Castanea* honey.

5.3.1.3. Absolute quantitative analysis

Absolute quantitative pollen analysis can shed light on over- or underrepresentation of pollen grains in a sample. Several methods can be applied to obtain the absolute number of pollen grains in honey. Typically, 10 g of honey material are analysed.

By adding known numbers of additional particles to the sample, estimations of the total pollen content can be made. *Lycopodium* spores have been used as quantitative markers for many decades (Stockmarr 1971; Bryant and Jones 2001). Recently, there have been attempts to use artificial products such as palynospheres, which are black ceramic microbeads with similar chemical and physical properties to sporopollenin (Kitaba and Nakagawa 2017). The method by Von der Ohe (2004) uses a vacuum filtration apparatus to filter the diluted honey, after which the filter paper is transferred onto a slide and the pollen is counted. Another method used by (Dinkov 2015) places diluted honey in a Bürker's chamber with two semi-reflective segments.

Standard melissopalynology typically only includes relative quantitative analysis of honey, which is sufficient for the identification geographical and botanical origin in routine application. Additionally, the absolute quantitative content of pollen in honey may be measured, e.g., when analysing the botanical origin of certain unifloral honeys. Different unifloral honey types display different absolute pollen concentrations, as in Demianowicz (1964) and Bryant & Jones (2001).

For this research project, no absolute quantitative measurements were performed. However, the pollen content of honey samples with a high percentage of *Castanea* pollen (e.g., the samples from Graz and Ljubljana) was notably higher than that of typical multifloral honey. On the other hand, samples with a high percentage of *Tilia* and *Robinia* pollen (e.g., from Vienna and Bratislava) had notably lower overall pollen concentrations, while samples with predominantly *Ailanthus altissima* and Rosaceae pollen had a medium overall pollen content. This is in line with previous studies (e.g., Bryant and Jones 2001; Von der Ohe et al. 2004).

5.3.1.4. Non-pollen palynomorphs

Honeydew elements such as fungal spores and algae were frequently found in hydrated urban honey samples. They were generally absent when the sample was

acetolysed. The presence of honeydew elements may point to active foraging of honeydew on deciduous trees such as *Quercus* sp., *Tilia* sp., *Acer* sp., and others known to be associated with phloem sapping aphids (Beckh and Camps 2009). Honeybees might feed on “urban” honeydew in periods of drought or nearby heavily built-upon sites, when the available plant nectar is limited. However, in a legal context, honey must only be labelled as honeydew honey when the origin of honeydew are coniferous trees such as *Pinus* sp., *Picea* sp., or *Abies* sp. (DLMBK 2023). There was no trace of combustion-related soot particles or elevated levels of microplastics in the analysed urban honey samples.

5.4. COMPLEMENTARY INTEGRATION OF OTHER METHODS

Several other microscopical and molecular methods can be used in a complementary fashion. For example, they may assist pollen identification through improved microscopical imaging and refining what parameters to search for. They might also lead to the identification of particular plant species from a group of very similar looking pollen types with the help of DNA-based methods. When applying multiple methods to one sample, the chain of possibility has to be considered, especially when the volume of material is limited. For example, acetolysed pollen cannot be further analysed with DNA metabarcoding, as all organic components of the cytoplast have been destroyed. From hydrated pollen, on the other hand, DNA may still be experimentally extracted and likewise acetolysis for LM and SEM may be performed.

In addition to LM analyses, the pooled honey samples from Chapter 4.B. were also examined with SEM. Rosaceae pollen types such as *Prunus* sp., *Prunus domestica*, *Malus* and *Pyrus* types, and *Rubus* species could be better distinguished (see Appendix, Figure A13). Further, the pollen wall of 3-colpate Lamiaceae species could more easily be distinguished from, e.g., *Veronica* sp. pollen. Pollen counting in SEM, as described by (Jones and Bryant 2007) for soil samples, has not been taken up in routine melissopalynological analysis.

The correct identification of “unknown” pollen types is essential when they occur in significant amounts, as they might point to a peculiar mass foraging resource. In the framework of establishing a DNA barcoding system for plants at the Austrian Agency

for Health and Food Safety (AGES), all 2020 honey samples from Vienna and all pooled honey samples from Chapter 4.B. were experimentally sequenced using the ITS2 primer system and the Galaxy-Pipeline customised database downloaded from NCBI. The aim was to increase the number of detected taxa and to identify pollen types to a lower taxonomic rank. For the Asteraceae, Brassicaceae, Ranunculaceae, and Rosaceae families, it was possible to refine the results from pollen analysis. With the blast search tool, it was further possible to identify *Sophora japonica* as a common pollen type in honey from Vienna (see Appendix, Table A1). Microscopically, the pollen of *Sophora japonica* (Appendix, Figure A14) is rather difficult to distinguish from other small reticulate tricolporate pollen types. Quantitatively, the percentages of *Sophora* pollen grains in LM and sequence reads after DNA metabarcoding were similar.

In contrast, some other pollen types with considerable concentrations in the microscopical analysis, such as *Gleditsia triacanthos* and *Parthenocissus* sp., did not appear in the results of DNA sequencing at all. Others were largely underrepresented, highly affecting the conclusion of the analysis. For example, *Acer* sp. occurred with 20.8% in LM, but less than 1% of processed sequence reads were appointed to various *Acer* species and hydrides combined (sample “1M_Munich”, see Chapter 4.B.). In sample “1M_Vienna”, *Ailanthus altissima* occurred with 27% in LM, but again less than 1% of all sequence reads. Also, *Castanea sativa* could only be detected in very little amounts of 1.3% of sequence reads, while comprising 40.8% of pollen grains in the LM analysis (sample “1M_Ljubljana”). On the other hand, pollen of several Rosaceae, Brassicaceae, and Fabaceae species was overrepresented in the spectrum of sequence reads. For example, *Trifolium repens* and closely related *Trifolium* species comprised 14% of sequence reads in sample “1M_Ljubljana”, but only 0.6% of counted pollen grains in LM. There have been multiple issues with the authenticity of taxonomic results as well. Botanical aggregates such as *Taraxacum* and *Rubus* were split into dozens of subspecies and hybrids by DNA sequencing. This shows the power of DNA-based identification methods, but is hardly relevant for routine honey analysis. There, they may be combined into genus types for better comprehensibility. Other taxa detected with DNA sequencing were consequently assigned the name of a species not plausible to occur in Central Europe, either natively or as ornamental, such as *Plantago ovata*

(Kew Royal Botanical Gardens 2025). Such challenges stress the need for competent botanical knowledge in the assessment of DNA-based results.

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6. CONCLUSION

This research highlights the specific challenges involved in the characterisation of urban honey and the identification of its geographical origins. It presents various methodological approaches for melissopalynological analyses and discusses their advantages and shortcomings. Pollen analysis is a powerful tool to identify the botanical diversity in urban honey and trace the foraging resources of urban honeybees. To this end, light microscopy proved to be a highly suitable approach.

The key findings of this research include the following:

- The high taxa richness found in urban honey reflects the diversity of heterogeneous urban green spaces, which are composed of semi-natural and ornamental, native and neophytic floral elements.
- The analysis of pollen spectra showed a strong footprint of local vegetation, which is often shaped by municipal planting regimes, ornamental trees, urban gardening patches, or a specific city topography.
- The intentional planting of climate-adapted and nectariferous species enhances the foraging resources of honeybees. Bee-friendly planting choices offer pollen with sufficient nutrients.
- Suburban apiary locations and locations in smaller cities showed some of the highest pollen diversities in honey. Foraging resource diversity increases when the 3 km flight radius of honeybees covers multiple ecotones and land use types.
- Abiotic parameters such as weather conditions are responsible for the majority of variation in honey from the same apiary location over several years.
- Through late flowering ornamental trees, the honeybee foraging season in urban areas is extended far into summer. This may concern urban beekeepers in planning the time and frequency of honey extraction.

Further focus areas of this research were the comparison of melissopalynological methods of honey preparation and analysis, as well as the benefit of reliable references to assist pollen identification. These are the main conclusions:

- Both the acetolysed and hydrated method for light microscopy are reliable when it comes to the identification of the most common pollen types. However, studies on the diversity of pollen should consider the hydrated method.

- The complementary use of additional melissopalynological methods such as DNA metabarcoding may provide benefits for the identification of most pollen types, but still lacks validation, universal standards, and quantitative accuracy.

- Open access online pollen databases are invaluable for melissopalynological analyses, especially when they comprise plants of worldwide origin and depict certain quality standards.

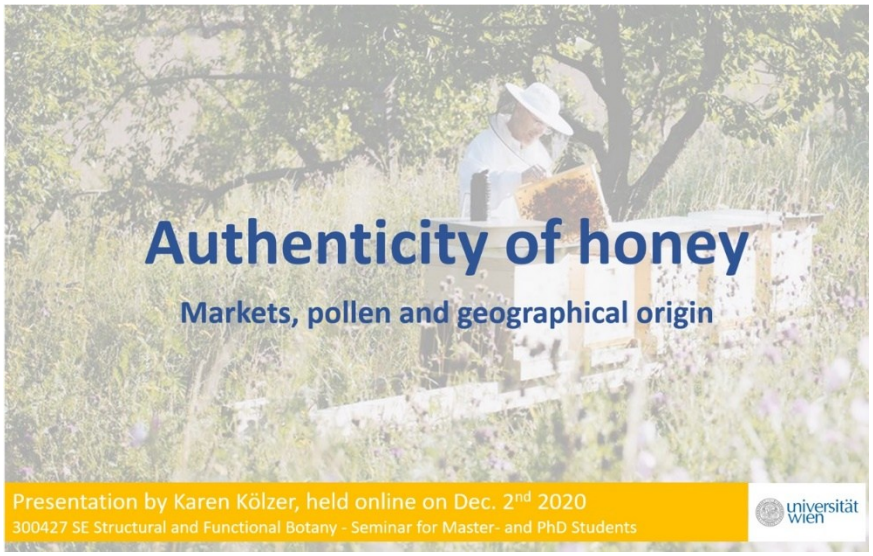
To sum up, this research shows that urban green spaces provide diverse floral sources for honeybees and that microscopical analysis methods are adequate to tackle challenges that may arise concerning the authenticity of urban honey. However, traditional standards for the labelling of geographical origin may not fully capture the realities of urban honey composition, which oftentimes points to multiple world regions within one honey sample. This suggests the need to revise regulatory frameworks in a way that accommodates urban-specific foraging patterns.

SUPPLEMENTS

I. TALKS

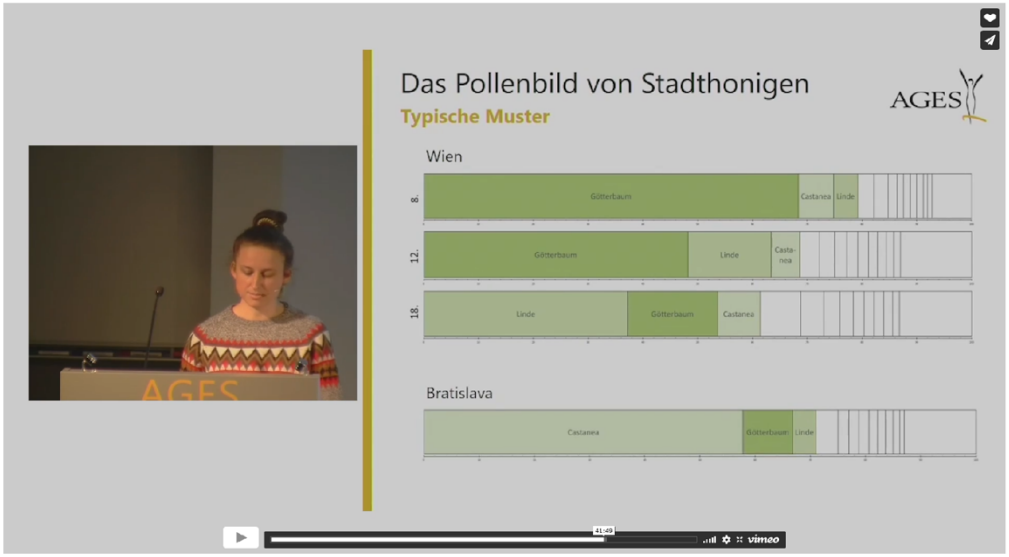
a) Seminar contribution, online

Title	Authenticity of honey – Markets, pollen and geographical origin
Topic	Focus of this talk was the importance of applied honey analysis for quality control on the global honey market, the beekeeping structure in Austria, and legal requirements regarding the botanical and geographical origin of honey.
Duration	45 minutes
Event	300427: Seminar for Master and PhD students of the Division for Structural and Functional Botany
Location	Department for Botany and Biodiversity Research, University of Vienna, Rennweg 14, 1030 Vienna, Austria (online)
Date	December 2, 2020



b) Lunchtime Learning, Vienna

Title	Urban honey: Surprises and challenges in the analysis of origin
Topic	This talk aimed at comparing pollen spectra from the first year of analysing urban honey. It highlighted the most intriguing pollen types and looked at various parameters which contribute to the differences in pollen diversity.
Duration	60 minutes
Event	“Lunchtime Learning”, a weekly lecture series for researchers and staff at the Austrian Agency for Health and Food Safety (AGES).
Location	Large lecture hall of the Austrian Agency for Health and Food Safety, Spargelfeldstraße 191, 1220 Vienna, Austria (and online via livestream)
Date	December 16, 2021



c) VDSEE Symposium, Vienna

Title	Authenticity of urban honey: Pollen diversity along the urban-suburban gradient
Topic	In this talk, pollen spectra from three years of honey analysis were compared, with the focus on Vienna as a Central European metropolis. A change in taxa composition from suburban to urban apiaries was discussed. The most important foraging plants were presented along with potential exotic “marker” pollen for urban honey.
Duration	20 minutes
Event	Symposium of the Vienna Doctoral School of Ecology and Evolution
Location	University of Vienna Biology Building (UBB), Djerassiplatz 1, 1030 Vienna, Austria
Date	February 16, 2024

universität wien

Vienna Doctoral School of Ecology and Evolution

16th February 2024

Certificate of Participation

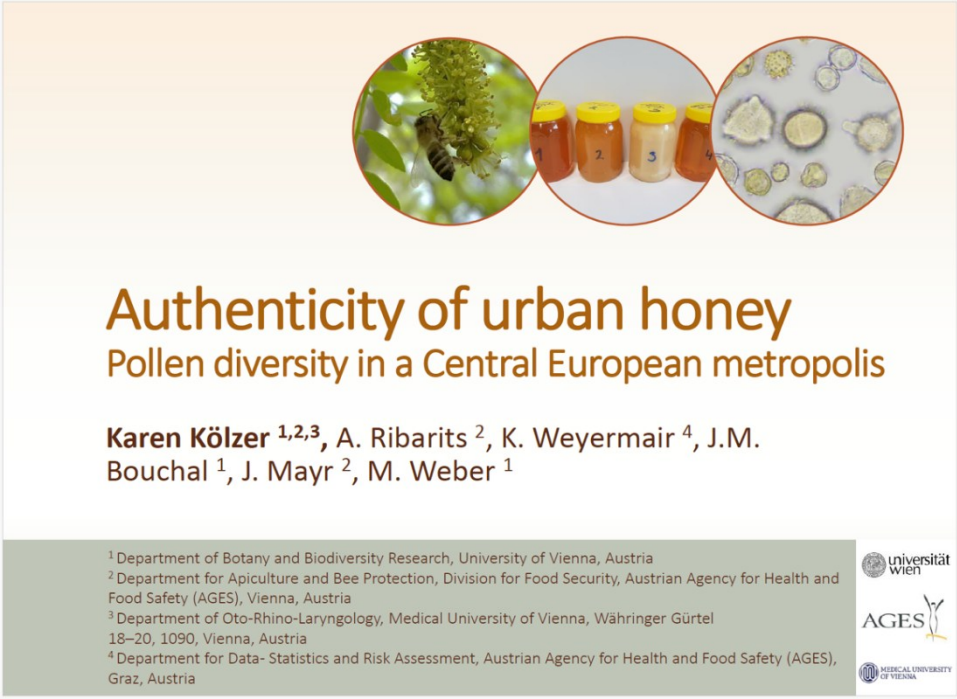
This is to certify that Karen Kölzer gave an **oral presentation** at the **Vienna Doctoral School of Ecology and Evolution Symposium 2024** that was held on the 16th February 2024 at the Universität Wien Biology Building.

Antonia Vogel

Antonia Vogel, PhD
VDSEE Executive Manager

d) IPC/IOPC Conference, Prague

Title	Authenticity of urban honey: Pollen diversity in a Central European metropolis
Topic	The most important foraging plants in Vienna were presented along with potential exotic "marker" pollen for urban honey. A change in taxa composition from suburban to urban apiaries was noted. Also the specific challenges of assessing urban honey and some of the surprise findings were discussed.
Duration	20 minutes
Event	15th International Palynological Congress and 11th International Organisation of Palaeobotany Conference
Location	Prague, Czech Republic
Date	May 28, 2024



II. ABSTRACTS

a) VDSEE Symposium, Vienna

market participation, more sustainable investments and less greenwashed investments in the incentivized investment decision, also among participants without investment experience. Participants with higher levels sustainable finance literacy are four times more likely to identify a greenwashed fund and have fewer misperceptions about sustainable investments. While sustainable finance literacy correlates with advanced financial literacy, we find significantly higher effect sizes and additional variance explained in the outcome variables compared to advanced financial literacy. Thus, sustainable finance literacy is a complementary concept to advanced financial literacy. The results allow to determine the importance of sustainable financial literacy for the sustainability transition. Based on the measurement instrument, academics and practitioners (e.g., for financial advisors) can adopt their financial advice to potential investors.

Karen Kölzer

Pollen diversity in honey from Apis mellifera along the urban-suburban gradient in a Central European metropolis

Karen Koelzer[1,2], Martina Weber[1], Alexandra Ribartis[2]

[1]Division for Structural and Functional Botany, Department of Botany and Biodiversity Research, University of Vienna, Austria; [2]Department for Apiculture and Bee Protection, Institute for Seed and Propagating Material, Phytosanitary Service and Apiculture, Division for Food Security, Austrian Agency for Health and Food Safety, Vienna, Austria.

Urban beekeeping has become a popular trend in many cities worldwide. Green spaces such as parks, private gardens, and road verges provide pollinators with diverse floral resources. Pollen analysis with light microscopy is a valuable tool to identify foraging plants of honeybees and allow to determine the botanical and geographical origin of honey. However, urban areas with their high diversity of ornamental, non-native plants pose a specific challenge in authenticity checks. Because they are also exceedingly affected by climate warming, many municipalities have started using adapted planting regimes to adapt to heat and drought.

With the aim to characterize the "urban", pollen in honey from eighteen apiaries across Vienna, Austria, was microscopically analysed over three consecutive years. Pollen grains were counted out to 500 per sample to determine the relative abundances of each pollen type. Differences in the pollen spectra were qualitatively and quantitatively examined according to year, extraction time and extent of urban built-up area within the typical 3 km flight radius of honeybees.

A total of 202 plant taxa could be identified, of which 48% to genus level and 25% to species level. The median number of pollen types per sample was 46. Overall, out of 71 identified ornamental plant taxa, 41 were non-native. Woody species were major contributors of pollen and nectar for urban honeybees, including the invasive Ailanthus altissima, which was the predominant pollen type in 15 out of 50 samples. Other non-native trees important for mass foraging included *Gleditsia triacanthos, Sophora japonica, Koelreuteria paniculata* and *Liriodendron tulipifera.*

This research evaluates bee-friendly municipal and private planting regimes in urban areas. The results suggest potential synergies between climate-adaptive taxa, pollinator ecology, and food security in cities.

b) IPC/IOPC Conference, Prague

28/05/2024, 17:00–19:00, Room: Leo
M08 Open symposium on basic (LM, SEM, TEM) and applied palynology

O-077 - Pollen diversity in honey from a Central European metropolis

Karen Koelzer[1], Alexandra Ribarits[2], Josef Mayr[2], Karin Weyermair[3], Johannes Martin Bouchal[1], Martina Weber[1]

1 University of Vienna, Botany and Biodiversity Research, Vienna, Austria
2 Austrian Agency for Health and Food Safety, Food Security, Vienna, Austria
3 Austrian Agency for Health and Food Safety, Data – Statistics and Risk Assessment, Vienna, Austria

Urban beekeeping has become a popular trend in many cities worldwide. Green spaces such as parks, private gardens, and road verges provide pollinators with diverse floral resources. Pollen analysis with light microscopy is a valuable tool to identify foraging plants of honeybees and determine honey's botanical and geographical origin. However, urban areas, with their high diversity of ornamental, non-native plants, pose a specific challenge in authenticity checks. Because they are also exceedingly affected by climate warming, many municipalities have started using adapted planting regimes to adapt to heat and drought.

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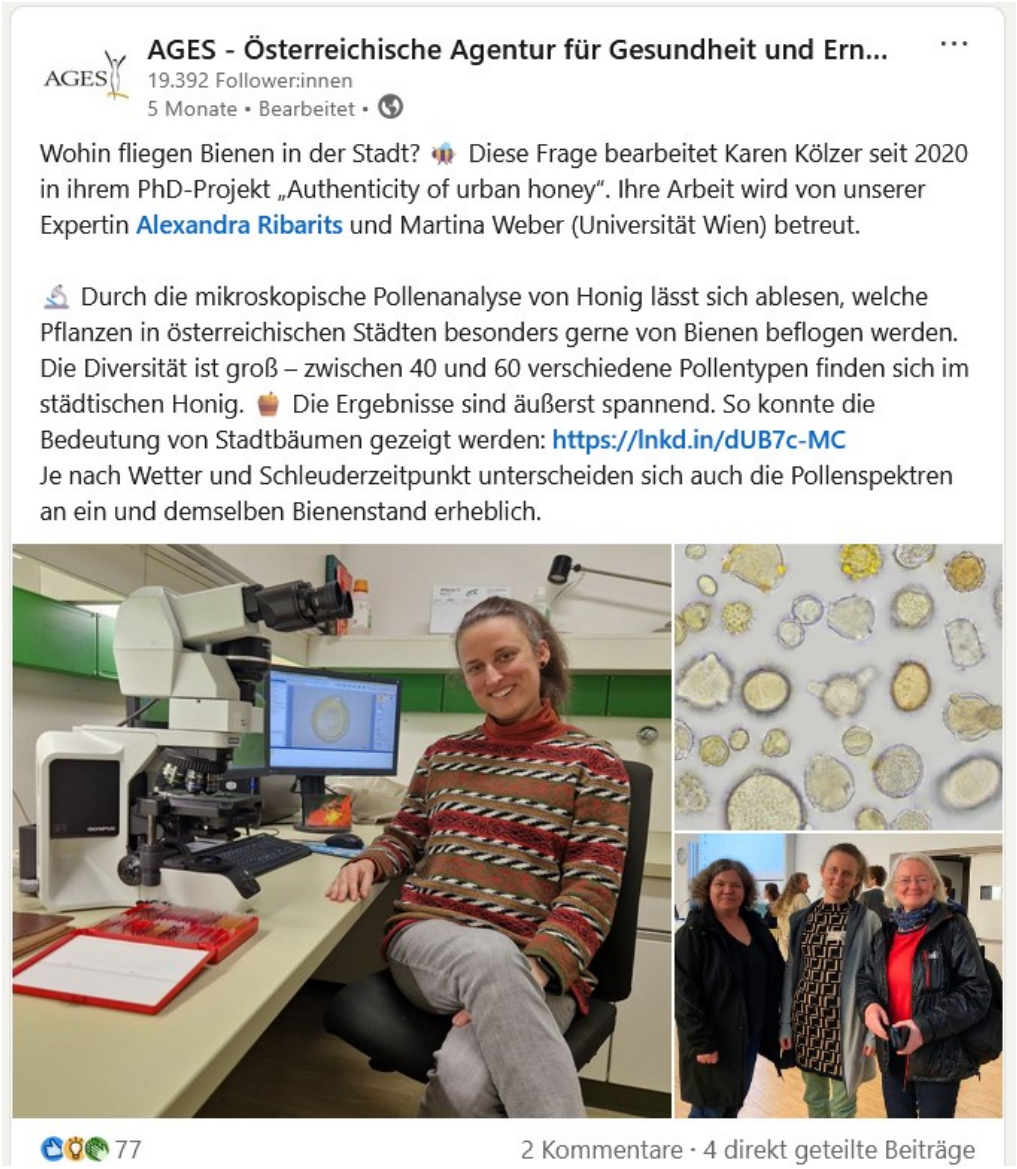
This research evaluates bee-friendly municipal and private planting regimes in urban areas. The results suggest potential synergies between climate-adaptive taxa, pollinator ecology, and food security in cities.

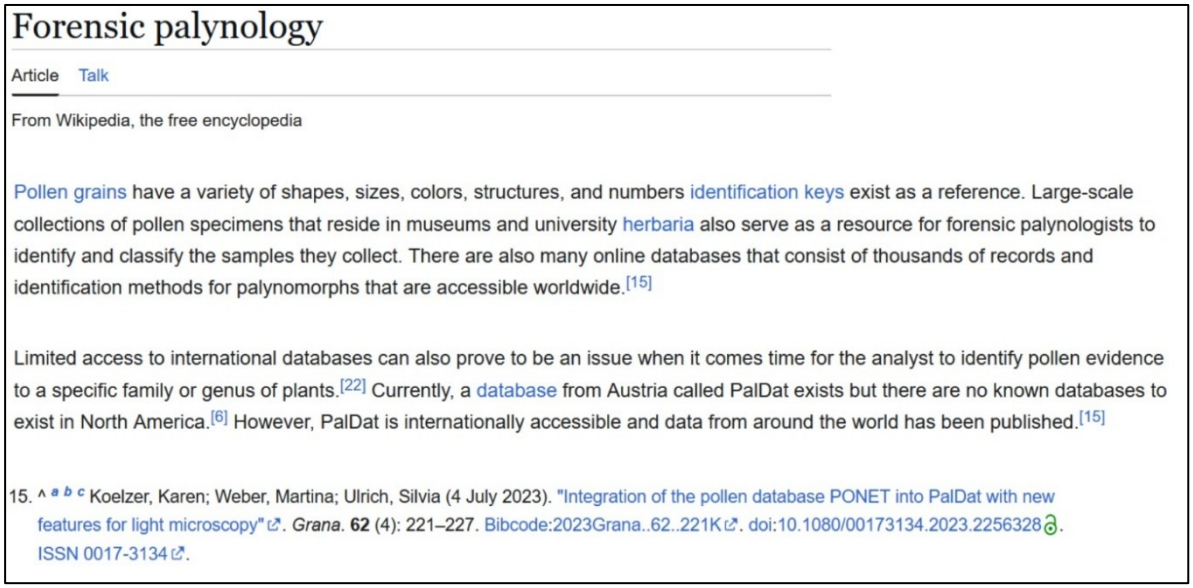
III. (SOCIAL) MEDIA

a) Facebook

b) LinkedIn


AGES - Österreichische Agentur für Gesundheit und Ern...
19.392 Follower:innen
5 Monate • Bearbeitet •
Wohin fliegen Bienen in der Stadt? Diese Frage bearbeitet Karen Kölzer seit 2020 in ihrem PhD-Projekt „Authenticity of urban honey“. Ihre Arbeit wird von unserer Expertin Alexandra Ribarits und Martina Weber (Universität Wien) betreut.
Durch die mikroskopische Pollenanalyse von Honig lässt sich ablesen, welche Pflanzen in österreichischen Städten besonders gerne von Bienen beflogen werden. Die Diversität ist groß – zwischen 40 und 60 verschiedene Pollentypen finden sich im städtischen Honig. Die Ergebnisse sind äußerst spannend. So konnte die Bedeutung von Stadtbäumen gezeigt werden: https://lnkd.in/dUB7c-MC
Je nach Wetter und Schleuderzeitpunkt unterscheiden sich auch die Pollenspektren an ein und demselben Bienenstand erheblich.
77
2 Kommentare · 4 direkt geteilte Beiträge

c) Wikipedia



Forensic palynology

Article Talk

From Wikipedia, the free encyclopedia

Pollen grains have a variety of shapes, sizes, colors, structures, and numbers identification keys exist as a reference. Large-scale collections of pollen specimens that reside in museums and university herbaria also serve as a resource for forensic palynologists to identify and classify the samples they collect. There are also many online databases that consist of thousands of records and identification methods for palynomorphs that are accessible worldwide.[15]

Limited access to international databases can also prove to be an issue when it comes time for the analyst to identify pollen evidence to a specific family or genus of plants.[22] Currently, a database from Austria called PalDat exists but there are no known databases to exist in North America.[6] However, PalDat is internationally accessible and data from around the world has been published.[15]

15. ^ a b c Koelzer, Karen; Weber, Martina; Ulrich, Silvia (4 July 2023). "Integration of the pollen database PONET into PalDat with new features for light microscopy". *Grana*. **62** (4): 221–227. Bibcode:2023Grana..62..221K. doi:10.1080/00173134.2023.2256328. ISSN 0017-3134.

APPENDIX

I. ANALYSED SAMPLES

a) Honey samples 2020

Sample	Country	City	Apiary Location	Latitude	Longitude	Harvest *	Extraction time	Picture
1H0-001	SVK	Bratislava	Búdková	48.1550365	17.0895079	0	Mid of July	A1-A
1H0-002	SVK	Bratislava	Cverna	48.1830859	17.1316538	0	Mid of July	A1-B
1H0-003	SVK	Bratislava	YIT	48.1481853	17.1314196	0	Mid of July	A1-C
1H0-004	AUT	Salzburg	Hotel Auersperg	47.8069475	13.0497235	0	Mid of July	A1-D
1H0-005	AUT	Salzburg	Kapuzinerberg	47.8051374	13.0586338	0	Mid of July	A1-E
1H0-007	AUT	Graz	Brückenkopf-gasse	47.0661665	15.4335418	0	Mid of July	A1-F
1H0-009	AUT	Innsbruck	Kapuzinerkloster	47.2697804	11.4011456	0	Mid of July	A1-G
1H0-010	AUT	Innsbruck	Paschbergweg	47.2561056	11.4216009	0	Mid of July	A1-H
1H0-011	AUT	Klagenfurt	Uni Campus	46.6171056	14.2661536	0	Mid of July	A1-I
1H0-012	AUT	Klagenfurt	Privatklinik Mariahilf	46.6274117	14.2964902	0	Mid of July	A1-J
1H0-013	AUT	Linz	Friedhof St. Barbara	48.2942658	14.3011517	0	Mid of July	A1-K
1H0-014	AUT	Linz	Mariendom	48.3002437	14.2860601	0	Start of July	A1-L
1H0-015	SLO	Ljubljana	SKB Headquarter	46.0534679	14.5049775	0	End of July	A1-M
1H0-016	SLO	Ljubljana	Urbani Čebelar	46.0716718	14.504031	0	End of July	A1-N
1H0-023	AUT	Bregenz	Vorkloster	47.4972818	9.71801167	0	End of July	A1-O
1H0-024	AUT	St. Poelten	HYPO NOE Headquarter	48.2019028	15.6335592	0	End of June	A1-P
1H0-025	AUT	Vienna	1., Stubenring	48.2098893	16.3831348	0	Start of July	A1-Q
1H0-026	AUT	Vienna	8., Palais Auersperg	48.2073	16.3546811	0	Start of July	A1-R
1H0-027	AUT	Vienna	12., Eichenstraße	48.1759247	16.336944	0	Start of July	A1-S
1H0-028	AUT	Vienna	15., Boutiquehotel	48.1991877	16.3337897	0	Start of July	A1-T
1H0-029	AUT	Vienna	18., BOKU Campus	48.2367494	16.3360584	0	Mid of July	A1-U
1H0-030	AUT	Vienna	22., NH Danube City	48.2353377	16.4219722	0	Start of July	A1-V
1H0-036	AUT	Vienna	10., Suchenwirtplatz	48.1700994	16.3735386	0	Mid of July	A1-W
1H0-037	AUT	Vienna	Airport	48.1239693	16.5580863	0	Mid of July	A1-X
1H0-041	AUT	Vienna	Donaufeld	48.2480645	16.4223233	0	Mid of July	A1-Y
1H0-038	AUT	Klagenfurt	Lakeside Park	46.6141754	14.2664902	0	End of July	A2-A
1H0-039	AUT	Vienna	6., Hotel ibis Mariahilf	48.1932452	16.3394863	0	End of July	A2-B
1H0-040	AUT	Vienna	14., Flötzersteig	48.2063154	16.2892644	0	End of July	A2-C
1H0-042	GER	Munich	Galopprennbahn	48.1484956	11.6761316	0	Mid of July	A2-D
1H1-017	GER	Munich	Nymphenburg	48.1620024	11.5002886	1	Mid of June	A2-E
1H1-018	GER	Munich	Schwabing	NA	NA	1	Mid of June	A2-F
1H1-019	GER	Munich	Nordfriedhof	48.1808231	11.6012688	1	Mid of June	A2-G
1H1-020	GER	Munich	Untermenzing	48.1665977	11.4592552	1	Mid of June	A2-H
1H1-021	GER	Munich	Forstenried	48.1090221	11.5003543	1	Mid of June	A2-I
1H1-022	GER	Munich	Feldkirchen	48.1465854	11.7323578	1	Mid of June	A2-J

Sample	Country	City	Apiary Location	Latitude	Longitude	Harvest *	Extraction time	Picture
1H1-031	AUT	Vienna	3., Hotel Daniel	48.1888621	16.3839276	1	Mid of June	A2-K
1H1-032	AUT	Vienna	15., KGV Schmelz	48.2032825	16.3232347	1	Mid of June	A2-L
1H1-033	AUT	Vienna	2., Prater Lusthaus	48.192547	16.4389394	1	Mid of June	A2-M
1H1-034	AUT	Vienna	23., Pappelteich	48.1453766	16.2470191	1	Mid of June	A2-N
1H1-035	CZE	Prague	Anděl	50.070966	14.4047154	1	Mid of June	A2-O
1H2-017	GER	Munich	Nymphenburg	48.1620024	11.5002886	2	End of July	A2-P
1H2-018	GER	Munich	Schwabing	NA	NA	2	End of July	A2-Q
1H2-019	GER	Munich	Nordfriedhof	48.1808231	11.6012688	2	End of July	A2-R
1H2-020	GER	Munich	Untermenzing	48.1665977	11.4592552	2	End of July	A2-S
1H2-031	AUT	Vienna	3., Hotel Daniel	48.1888621	16.3839276	2	End of July	A2-T
1H2-032	AUT	Vienna	15., KGV Schmelz	48.2032825	16.3232347	2	End of July	A2-U
1H2-033	AUT	Vienna	2., Prater Lusthaus	48.192547	16.4389394	2	End of July	A2-V
1H2-034	AUT	Vienna	23., Pappelteich	48.1453766	16.2470191	2	End of July	A2-W
1H2-035	CZE	Prague	Anděl	50.070966	14.4047154	2	End of July	A2-X

* indicates the spring harvest (1), summer harvest (2), or a singular honey extraction per year (0)

Figure A1: Original honey jars received for pollen analysis from the year 2020, part I.

Figure A2: Original honey jars received for pollen analysis from the year 2020, part II.

b) Honey samples 2021

Sample	Country	City	Apiary Location	Latitude	Longitude	Harvest *	Extraction time	Picture
2H0-004	AUT	Salzburg	Hotel Auersperg	47.8069475	13.0497235	0	End of July	A3-A
2H0-005	AUT	Salzburg	Kapuzinerberg	47.8051374	13.0586338	0	End of July	A3-B
2H0-007	AUT	Graz	Brückenkopf-gasse	47.0661665	15.4335418	0	Mid of July	A3-C
2H0-009	AUT	Innsbruck	Kapuzinerkloster	47.2697804	11.4011456	0	Mid of July	A3-D
2H0-010	AUT	Innsbruck	Paschbergweg	47.2561056	11.4216009	0	Mid of July	A3-E
2H0-013	AUT	Linz	Friedhof St. Barbara	48.2942658	14.3011517	0	Mid of July	A3-F
2H0-015	SLO	Ljubljana	SKB Headquarters	46.0534679	14.5049775	0	End of July	A3-G
2H0-016	SLO	Ljubljana	Urbani Čebelar	46.0716718	14.504031	0	End of July	A3-H
2H0-017	GER	Munich	Nymphenburg	48.1620024	11.5002886	0	End of July	A3-I
2H0-020	GER	Munich	Untermenzing	48.1665977	11.4592552	0	Mid of August	A3-J
2H0-019	GER	Munich	Nordfriedhof	48.1808231	11.6012688	0	End of August	A3-K
2H0-021	GER	Munich	Forstenried	48.1090221	11.5003543	0	End of August	A3-L
2H0-042	GER	Munich	Galopprennbahn	48.1484956	11.6761316	0	Mid of July	A3-M
2H0-050	GER	Munich	Gauting	48.0696372	11.3782847	0	End of May	A3-N
2H0-024	AUT	St. Poelten	HYPO NOE Headquarters	48.2019028	15.6335592	0	End of June	A3-O
2H0-025	AUT	Vienna	1., Stubenring	48.2098893	16.3831348	0	Mid of July	A3-P
2H0-026	AUT	Vienna	8., Palais Auersperg	48.2073	16.3546811	0	Mid of July	A3-Q
2H0-027	AUT	Vienna	12., Eichenstraße	48.1759247	16.336944	0	Mid of July	A3-R
2H0-028	AUT	Vienna	15., Boutiquehotel	48.1991877	16.3337897	0	Mid of July	A3-S
2H0-029	AUT	Vienna	18., BOKU Campus	48.2367494	16.3360584	0	Mid of July	A3-T
2H0-034	AUT	Vienna	Pappelteich	48.1453766	16.2470191	0	Mid of July	A3-U
2H0-036	AUT	Vienna	10., Suchenwirtplatz	48.1700994	16.3735386	0	Mid of July	A3-V
2H0-037	AUT	Vienna	Airport	48.1239693	16.5580863	0	Mid of July	A3-W
2H0-038	AUT	Klagenfurt	Lakeside Park	46.6141754	14.2664902	0	End of July	A3-X
2H0-039	AUT	Vienna	6., Hotel ibis Mariahilf	48.1932452	16.3394863	0	Mid of July	A3-Y
2H0-044	AUT	Vienna	4., TU Campus	48.1985951	16.3685456	0	Mid of July	A4-A
2H0-045	AUT	Vienna	11., Gärtnerei	48.174473	16.4463359	0	Mid of July	A4-B
2H0-046	AUT	Vienna	20., Donaukanal	48.22651	16.3694662	0	Mid of July	A4-C
2H0-051	SVK	Bratislava	Presidential Palace	48.1493677	17.1077694	0	Start of September	A4-D
2H0-052	SVK	Bratislava	Government's Office	48.1525983	17.1094034	0	End of September	A4-E
2H0-053	SVK	Bratislava	Ministry of Defense	48.1654123	17.1303599	0	Mid of August	A4-F
2H0-054	SVK	Bratislava	Búdková	48.1550365	17.0895079	0	Mid of September	A4-G
2H0-055	SVK	Bratislava	Pan-European University	48.1591399	17.1642668	0	Mid of August	A4-H
2H0-056	SVK	Bratislava	Slovenská sporiteľňa	48.1676295	17.1504243	0	Mid of August	A4-I
2H0-057	SVK	Bratislava	CBC 5	48.1508585	17.1261641	0	End of August	A4-J

Sample	Country	City	Apiary Location	Latitude	Longitude	Harvest *	Extraction time	Picture
2H0-058	SVK	Bratislava	ČSOB Headquarters	48.1409453	17.095049	0	Start of September	A4-K
2H1-031	AUT	Vienna	3., Hotel Daniel	48.1888621	16.3839276	1	End of June	A4-L
2H1-032	AUT	Vienna	15., KGV Schmelz	48.2032825	16.3232347	1	End of June	A4-M
2H1-033	AUT	Vienna	2., Prater Lusthaus	48.192547	16.4389394	1	End of June	A4-N
2H1-040	AUT	Vienna	14., Flötzersteig	48.2063154	16.2892644	1	End of June	A4-O
2H2-031	AUT	Vienna	3., Hotel Daniel	48.1888621	16.3839276	2	Start of August	A4-P
2H2-032	AUT	Vienna	15., KGV Schmelz	48.2032825	16.3232347	2	Start of August	A4-Q
2H2-033	AUT	Vienna	2., Prater Lusthaus	48.192547	16.4389394	2	Start of August	A4-R
2H1-040	AUT	Vienna	14., Flötzersteig	48.2063154	16.2892644	2	Start of August	A4-S
2H1-035	CZE	Prague	Anděl	50.070966	14.4047154	1	End of June	A4-T
2H2-035	CZE	Prague	Anděl	50.070966	14.4047154	2	End of July	A4-U

* indicates the spring harvest (1), summer harvest (2), or a singular honey extraction per year (0)

Figure A3: Original honey jars received for pollen analysis from the year 2021, part I.

Figure A4: Original honey jars received for pollen analysis from the year 2021, part II.

c) Honey samples 2022

Sample	Country	City	Apiary Location	Latitude	Longitude	Harvest *	Extraction time	Picture
3H0-004	AUT	Salzburg	Hotel Auersperg	47.8069475	13.0497235	0	Mid of July	A5-A
3H0-005	AUT	Salzburg	Kapuzinerberg	47.8051374	13.0586338	0	Mid of July	A5-B
3H0-015	SLO	Ljubljana	SKB Headquarters	46.0534679	14.5049775	0	End of July	A5-C
3H0-016	SLO	Ljubljana	Urbani Čebelar	46.0716718	14.504031	0	Start of August	A5-D
3H0-025	AUT	Vienna	1., Stubenring	48.2098893	16.3831348	0	Mid of July	A5-E
3H0-026	AUT	Vienna	8., Palais Auersperg	48.2073	16.3546811	0	Mid of July	A5-F
3H0-027	AUT	Vienna	12., Eichenstraße	48.1759247	16.336944	0	Mid of July	A5-G
3H0-028	AUT	Vienna	15., Boutiquehotel	48.1991877	16.3337897	0	Mid of July	A5-H
3H0-029	AUT	Vienna	18., BOKU Campus	48.2367494	16.3360584	0	Mid of July	A5-I
3H0-030	AUT	Vienna	22., NH Danube City	48.2353377	16.4219722	0	Mid of July	A5-J
3H0-036	AUT	Vienna	10., Suchenwirtplatz	48.1700994	16.3735386	0	Mid of July	A5-K
3H0-037	AUT	Vienna	Airport	48.1239693	16.5580863	0	Mid of July	A5-L
3H0-044	AUT	Vienna	4., TU Campus	48.1985951	16.3685456	0	Mid of July	A5-M
3H0-045	AUT	Vienna	11., Gärtnerei	48.174473	16.4463359	0	Mid of July	A5-N
3H0-046	AUT	Vienna	20., Donaukanal	48.22651	16.3694662	0	Mid of July	A5-O
3H1-009	AUT	Innsbruck	Kapuzinerkloster	47.2697804	11.4011456	1	Start of June	A5-P
3H1-010	AUT	Innsbruck	Paschbergweg	47.2561056	11.4216009	1	Start of June	A5-Q
3H2-009	AUT	Innsbruck	Kapuzinerkloster	47.2697804	11.4011456	2	Start of August	A5-R
3H2-010	AUT	Innsbruck	Paschbergweg	47.2561056	11.4216009	2	Start of August	A5-S

* indicates the spring harvest (1), summer harvest (2), or a singular honey extraction per year (0)

Figure A5: Original honey jars received for pollen analysis from the year 2022.

II. ADDITIONAL RESULTS

The following table indicates the number of analysed samples per city (in brackets) and in how many of these a certain pollen type was found.

Pollen Type	Bratislava (11)	Bregenz (1)	Graz (2)	Innsbruck (8)	Klagenfurt (4)	Linz (3)	Ljubljana (6)	Munich (17)	Prague (4)	Salzburg (6)	St. Poelten (2)	Vienna (50)	in n samples (of 114 total)
Abelia sp.	-	-	-	-	-	-	-	1	-	-	-	-	1
Acer negundo	1	-	1	2	-	-	-	1	-	-	-	2	7
Acer sp.	11	1	2	7	4	3	6	17	4	5	2	46	108
Achillea-Type	1	-	-	7	4	2	3	8	2	2	1	5	35
Aesculus hippocastanum	11	1	2	8	3	3	6	17	4	4	2	48	109
Aesculus x *carnea*	2	1	2	5	2	1	1	3	1	4	2	19	43
Agrimonia sp.	1	-	-	5	1	-	2	5	1	3	-	8	26
Ailanthus altissima	11	-	2	7	4	3	6	9	4	6	2	49	103
Allium sp.	6	1	2	3	2	2	2	13	3	3	1	30	68
Alnus sp.	-	-	-	2	2	1	-	5	-	-	1	6	17
Alpinia sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Amaranthaceae	5	-	1	3	1	-	3	7	2	-	-	8	30
Ambrosia sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Amorpha fruticosa	7	-	-	1	-	-	2	5	3	4	1	14	37
Anchusa sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Anemone-Type	10	1	1	8	1	2	4	17	-	6	2	39	91
Anthericum-Type	-	-	-	-	-	-	-	-	-	-	-	1	1
Anthyllis sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Apiaceae	2	1	1	6	2	-	3	8	2	4	1	19	49
Artemisia sp.	4	-	1	5	-	-	1	6	2	1	-	6	26
Aruncus dioicus	-	-	2	7	3	1	5	12	2	2	2	10	46
Asparagus sp.	1	-	-	1	-	-	2	1	2	-	-	5	12
Asteraceae	4	-	1	5	2	2	6	13	2	5	-	12	52
Astragalus sp.	1	-	-	1	-	-	1	4	1	5	1	9	23
Atropa belladonna	-	-	-	1	-	-	-	-	-	-	-	1	2
Begonia sp.	4	1	-	5	2	3	1	8	2	6	1	28	61
Bellis-Type	1	-	-	5	-	-	2	1	-	1	-	6	16
Berberidaceae	1	-	-	2	-	1	-	-	1	-	-	6	11
Betula sp.	4	-	-	3	1	1	2	12	3	2	1	20	49
Boraginaceae	-	-	-	-	-	-	-	2	-	-	-	3	5
Brassica sp.	8	1	-	2	1	2	-	10	4	1	2	13	44
Brassicaceae	9	-	-	7	2	3	4	16	4	2	2	36	85
Brassicaceae_small	-	-	-	-	-	-	-	-	-	-	-	1	1
Brassiceae_large	-	-	-	-	-	-	-	-	-	-	-	1	1

Pollen Type	Bratislava (11)	Bregenz (1)	Graz (2)	Innsbruck (8)	Klagenfurt (4)	Linz (3)	Ljubljana (6)	Munich (17)	Prague (4)	Salzburg (6)	St. Poelten (2)	Vienna (50)	in n samples (of 114 total)
Bryonia dioica	-	-	-	-	-	-	-	-	1	-	1	-	2
Buddleja sp. (4-aperturate)	3	-	1	8	-	-	3	10	1	3	-	29	58
Bupleurum sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Buxus sempervirens	-	1	-	1	-	-	-	1	-	1	-	4	8
Campanula-Type	1	-	-	1	-	1	1	4	1	1	-	9	19
Caprifoliaceae	-	-	1	-	-	-	1	-	-	1	-	2	5
Capsicum sp.	-	-	-	-	-	-	-	-	-	-	-	2	2
Carpinus betulus	2	-	-	1	-	-	-	5	-	1	-	3	12
Caryophyllaceae	1	-	1	5	2	1	3	4	-	4	-	7	28
Caryophyllaceae_large	-	-	-	1	-	-	1	-	-	-	-	-	2
Caryophyllaceae_small	-	-	-	-	-	-	1	-	-	-	-	-	1
Castanea sativa	11	-	2	8	4	3	6	9	3	6	2	47	101
Catalpa sp.	6	-	-	-	1	-	3	1	-	1	-	8	20
Celtis sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Centaurea jacea-Type	-	-	-	1	3	-	2	7	-	2	-	2	17
Centaurea scabiosa	-	-	-	-	-	-	-	1	-	-	-	1	2
Centaurium sp.	4	-	1	1	-	-	-	4	-	1	-	5	16
Cephalaria-Type	-	-	-	-	-	-	-	-	-	-	-	1	1
Cestrum sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Cirsium-Type	3	-	-	3	1	-	1	5	2	-	-	11	26
Citrullus lanatus	-	-	-	1	-	-	1	-	-	-	-	-	2
Citrus sp.	-	-	-	-	-	-	1	-	-	-	-	1	2
Cleome sp.	-	-	-	-	-	-	-	1	-	-	-	1	2
Consolida sp.	-	-	-	1	-	-	-	-	-	-	-	-	1
Convolvulaceae	-	-	-	-	-	-	-	-	1	-	-	-	1
Convolvulus sp.	1	-	-	-	-	-	-	1	1	2	-	4	9
Cornus florida	-	-	-	1	1	-	-	1	-	-	1	2	6
Cornus mas	-	-	-	-	-	-	-	1	-	-	-	6	7
Cornus sanguinea	8	-	2	8	4	3	4	15	3	6	1	36	90
Cornus sp.	-	-	-	-	-	-	-	-	1	-	-	-	1
Corydalis sp.	1	-	-	-	-	-	1	-	-	-	-	-	2
Corylus avellana	-	1	-	-	2	-	-	4	-	1	1	3	12
Corylus colurna	-	-	-	1	1	-	3	3	-	-	-	6	14
Cotinus coggygria	-	-	-	-	-	-	1	1	-	-	-	3	5
Crocus sp.	-	-	-	-	-	-	2	-	-	-	-	-	2
Cucumis melo	-	-	-	-	-	-	1	-	-	-	-	-	1
Cucurbita pepo	-	-	-	-	-	-	1	-	-	-	-	-	1
Cupressaceae	-	1	-	2	-	-	-	4	-	1	-	4	12
Cuscuta sp.	-	-	-	-	-	-	1	-	-	-	-	-	1

Pollen Type	Bratislava (11)	Bregenz (1)	Graz (2)	Innsbruck (8)	Klagenfurt (4)	Linz (3)	Ljubljana (6)	Munich (17)	Prague (4)	Salzburg (6)	St. Poelten (2)	Vienna (50)	in n samples (of 114 total)
Cyanus segetum	-	-	-	-	-	-	-	3	-	1	-	9	13
Cyclamen-Type	1	-	-	2	4	1	1	2	1	2	1	15	30
Cynoglossum sp.	-	1	-	-	-	-	-	2	-	-	-	8	11
Cyperaceae	1	1	-	3	2	-	-	1	-	2	-	2	12
Dasiphora sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Davidia involucrata	-	-	-	-	1	1	-	-	-	-	-	1	3
Echium sp.	11	-	1	2	2	2	2	11	2	1	2	31	67
Elaeagnus angustifolia	5	-	-	1	-	-	-	1	-	-	1	14	22
Erica sp.	-	-	-	3	-	-	-	1	-	-	1	1	6
Erodium sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Euonymus sp.	-	-	-	1	-	-	-	-	-	-	1	2	4
Euphorbia sp.	1	-	1	-	-	1	-	3	-	-	2	-	8
Exacum affine	-	-	-	3	1	-	-	2	-	1	-	1	8
Fabaceae	-	-	1	1	-	1	-	4	-	-	-	5	12
Fagopyrum esculentum	-	-	-	-	2	-	1	3	-	-	-	-	6
Fagus sylvatica	1	-	-	-	-	-	-	-	-	-	-	1	2
Fallopia sp.	-	-	-	-	-	-	-	-	-	-	-	2	2
Filipendula sp.	-	-	1	5	4	-	4	7	-	6	1	9	37
Fontanesia sp.	-	-	-	-	-	-	-	-	-	-	-	4	4
Fragaria sp./*Potentilla* sp.	5	1	2	4	4	2	5	12	2	6	2	32	77
Frangula alnus	1	-	-	5	4	1	2	5	-	3	-	9	30
Fraxinus excelsior	4	1	-	2	2	1	1	12	2	1	-	6	32
Fraxinus ornus	5	-	2	7	3	1	3	2	2	3	-	20	48
Fraxinus sp.	-	-	-	-	-	-	-	1	-	-	-	2	3
Gagea sp.	-	-	-	1	-	-	-	-	-	-	-	-	1
Galium-Type	-	-	1	1	-	-	-	2	-	1	1	5	11
Genista sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Gentiana sp.	-	-	-	2	-	-	-	-	-	-	-	-	2
Geranium sp.	2	-	-	-	1	-	-	-	-	1	1	5	10
Geum sp.	-	-	-	1	-	-	-	3	-	2	1	2	9
Ginkgo biloba	-	-	-	-	-	-	-	2	-	-	-	4	6
Gleditsia triacanthos	8	-	2	7	4	3	6	10	4	4	2	47	97
Hamamelidaceae	-	-	-	-	-	-	-	-	-	-	-	1	1
Hedera helix	4	1	2	6	4	-	4	7	3	1	1	15	48
Hedychium sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Helianthemum sp.	6	-	-	6	1	-	3	3	-	4	-	10	33
Helianthus sp.	4	-	-	1	-	-	1	2	1	1	-	3	13
Heracleum sp.	-	-	-	3	-	-	1	1	-	3	1	1	10
Hibiscus sp.	-	-	-	-	-	-	1	-	-	-	-	-	1

Pollen Type	**Bratislava (11)**	**Bregenz (1)**	**Graz (2)**	**Innsbruck (8)**	**Klagenfurt (4)**	**Linz (3)**	**Ljubljana (6)**	**Munich (17)**	**Prague (4)**	**Salzburg (6)**	**St. Poelten (2)**	**Vienna (50)**	**in n samples (of 114 total)**
Hyacinthaceae	-	-	-	-	-	-	-	3	-	-	1	-	4
Hydrangea sp.	-	-	-	-	-	-	1	1	-	-	2	-	4
Hypericum sp.	2	-	-	2	-	1	1	8	1	5	1	16	37
Ilex aquifolia	3	1	1	2	-	1	3	2	-	1	-	6	20
Ilex-Type	-	-	-	2	-	-	-	-	-	-	-	1	3
Impatiens sp.	-	-	-	4	4	-	2	7	-	1	1	3	22
Iridaceae	-	-	-	-	-	-	1	1	-	-	-	3	5
Iris sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Jasminum nudiflorum	-	-	-	-	-	-	-	-	-	-	-	1	1
Juglans sp.	-	-	-	1	-	-	-	-	-	-	-	6	7
Juncus sp./*Luzula* sp.	1	-	-	5	1	-	-	-	-	-	-	1	8
Knautia sp.	-	-	-	-	-	-	2	-	-	-	-	3	5
Koelreuteria paniculata	7	-	1	4	-	-	-	2	-	-	-	38	52
Lamiaceae-Type (3-aperturate)	5	-	1	1	-	-	4	6	1	-	2	7	27
Lamiaceae-Type (6-aperturate)	1	1	2	4	4	-	2	8	1	4	-	25	52
Lathyrus sp.	1	-	1	-	-	-	-	1	-	2	-	6	11
Leucojum sp.	-	-	-	-	-	-	1	-	-	-	-	-	1
Ligustrum vulgare	-	-	-	1	-	-	-	2	-	-	-	6	9
Liliaceae	-	-	-	1	-	-	1	-	-	1	-	1	4
Lilium sp.	-	-	-	1	-	-	-	-	-	2	-	1	4
Linum sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Liquidambar styraciflua	-	-	-	-	-	-	-	-	-	-	-	1	1
Liriodendron tulipifera	2	-	2	8	4	1	3	4	1	4	2	25	56
Lonicera sp.	2	1	-	6	1	2	4	6	-	2	1	12	37
Loranthus europaeus	2	-	-	-	-	-	-	-	-	-	-	10	12
Lotus sp.	1	-	-	1	3	1	5	6	-	1	-	6	24
Lysimachia-Type	-	-	-	-	-	-	-	3	-	-	-	-	3
Lythrum salicaria	1	-	-	-	1	-	1	1	1	-	-	2	7
Magnolia sp.	1	-	-	-	-	-	-	-	-	-	-	-	1
Malus-Type	11	1	2	7	3	3	6	14	4	3	2	44	100
Malva-Type	-	-	-	1	-	-	2	1	-	1	-	-	5
Medicago sp.	-	-	-	-	-	-	-	1	1	1	-	1	4
Melilotus sp.	5	-	-	2	1	1	3	5	2	4	-	12	35
Mercurialis sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Morus sp.	-	-	1	2	-	-	5	1	-	-	-	11	20
Muscari sp.	3	-	-	-	1	-	1	4	2	-	2	8	21
Myosotis sp.	6	1	1	6	3	3	5	12	3	3	-	32	75
Myrtaceae	-	-	-	1	-	-	-	-	-	-	-	-	1
Narcissus sp.	-	-	-	-	-	-	-	-	-	-	-	1	1

Pollen Type	**Bratislava (11)**	**Bregenz (1)**	**Graz (2)**	**Innsbruck (8)**	**Klagenfurt (4)**	**Linz (3)**	**Ljubljana (6)**	**Munich (17)**	**Prague (4)**	**Salzburg (6)**	**St. Poelten (2)**	**Vienna (50)**	**in n samples (of 114 total)**
Nicotiana sp.	-	-	-	2	1	-	1	4	-	-	-	5	13
Ocimum basilicum	-	-	-	-	-	-	-	1	-	1	-	-	2
Oleaceae	-	-	-	-	-	-	-	-	-	1	-	1	2
Omphalodes sp.	-	-	-	3	1	1	-	6	1	2	-	10	24
Onobrychis sp.	-	-	1	2	1	-	-	-	1	-	-	8	13
Orobanche sp.	-	-	-	-	-	-	-	-	1	1	-	-	2
Papaver sp.	6	-	1	4	2	1	-	7	1	5	1	22	50
Parthenocissus sp.	10	-	2	8	4	3	6	13	2	6	2	47	103
Paulownia tomentosa	-	-	-	-	-	-	-	-	-	-	-	2	2
Petunia sp.	3	1	1	6	3	1	4	6	2	4	1	18	50
Peucedanum-Type	-	-	-	-	-	-	-	1	-	-	-	-	1
Phacelia tanacetifolia	7	-	1	4	-	-	1	14	3	4	2	15	51
Phaseolus sp.	1	-	-	-	1	-	-	-	-	-	-	1	3
Picea sp./*Abies* sp.	-	1	-	1	3	-	1	6	2	1	1	6	22
Pinus sp.	4	-	2	7	4	-	1	6	1	2	2	24	53
Plantago sp.	11	1	1	8	4	3	6	15	3	6	2	46	106
Platanus acerifolia	2	-	-	2	1	2	-	-	1	-	-	20	28
Poaceae	10	1	2	8	4	2	4	14	3	6	1	37	92
Podostemaceae	-	-	-	-	-	-	-	-	1	-	-	1	2
Populus sp.	1	-	-	-	-	-	-	1	1	-	-	3	6
Primula sp.	1	-	-	-	-	-	-	-	-	-	-	1	2
Primula veris-Type	-	-	-	1	-	-	-	2	-	-	-	-	3
Prunus domestica	6	-	-	1	-	-	1	2	2	1	2	6	21
Prunus padus	3	-	-	-	-	-	-	1	-	-	1	-	5
Prunus sp.	9	1	1	7	4	2	6	14	4	4	2	33	87
Ptelea trifoliata	-	-	-	-	-	-	-	-	-	-	-	1	1
Pterocarya fraxinifolia	-	-	-	-	-	-	-	1	-	-	-	2	3
Quercus sp.	3	-	-	6	3	2	4	7	3	3	1	20	52
Reseda sp.	4	-	-	-	-	-	-	2	1	-	2	10	19
Rhamnus sp.	2	-	-	3	-	-	2	2	-	1	2	17	29
Rhinanthus sp.	-	-	-	-	-	1	-	-	-	-	-	-	1
Rhododendron sp./*Vaccinium* sp.	-	-	-	2	-	-	-	1	-	-	-	1	4
Ribes sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Robinia pseudoacacia	10	-	-	5	2	2	3	4	2	1	1	27	57
Rosaceae	4	-	-	7	1	1	3	5	1	2	1	27	52
Rubus sp.	11	1	-	8	4	3	5	14	4	6	2	34	92
Rumex sp.	2	-	2	5	2	-	1	8	1	2	-	16	39
Ruta graveolens	-	-	-	-	-	-	-	-	-	-	1	-	1
Salix purpurea-Type	-	-	1	4	-	-	2	1	-	5	1	11	25

Pollen Type	**Bratislava (11)**	**Bregenz (1)**	**Graz (2)**	**Innsbruck (8)**	**Klagenfurt (4)**	**Linz (3)**	**Ljubljana (6)**	**Munich (17)**	**Prague (4)**	**Salzburg (6)**	**St. Poelten (2)**	**Vienna (50)**	**in n samples (of 114 total)**
Salix sp.	10	1	2	8	4	3	6	15	4	6	2	34	95
Salvia glutinosa-Type	-	-	1	-	-	-	-	-	-	-	-	1	2
Salvia sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Sambucus-Type	10	1	2	7	4	2	3	18	3	6	2	58	116
Sanguisorba minor	-	-	-	-	-	-	-	-	-	-	-	1	1
Sanguisorba officinalis	-	-	-	-	-	-	-	-	-	1	-	2	3
Saussurea sp./*Jurinea* sp.	-	-	-	-	-	-	-	1	1	-	-	-	2
Saxifraga sp.	-	-	-	-	-	1	-	1	-	-	-	-	2
Scabiosa sp.	-	-	-	-	1	-	1	-	-	-	-	2	4
Scrophulariaceae	-	-	-	-	-	-	-	2	-	-	-	6	8
Sedum sp.	4	-	1	6	1	1	-	11	3	3	-	17	47
Sinapis sp./*Cardamine* sp.	2	-	1	2	2	2	1	4	1	-	1	8	24
Smyrnium perfoliatum	-	-	-	-	-	-	-	1	-	-	-	5	6
Solanaceae	2	1	-	4	-	1	1	3	-	1	-	4	17
Solanaceae_large	-	-	-	-	1	-	-	-	-	-	-	1	2
Solanum lycopersicum	-	-	-	-	-	-	-	-	-	-	-	1	1
Solanum sp.	-	-	-	-	-	-	-	-	1	-	-	-	1
Sophora japonica	6	-	-	2	1	-	4	2	1	1	-	28	45
Sorbus sp.	-	-	-	-	-	-	-	1	-	-	-	1	2
Spiraea sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Symphoricarpos sp.	-	-	-	1	-	-	-	3	-	-	-	4	8
Syringa vulgris	10	-	2	7	3	3	4	10	2	6	1	47	95
Tamarix sp.	-	-	-	-	-	-	-	-	-	-	-	3	3
Taraxacum-Type	6	1	2	8	3	3	6	12	3	4	1	28	77
Taxus-Type	3	1	-	1	-	1	-	6	2	1	-	11	26
Tetradium danielii	-	-	-	3	1	-	2	-	-	-	-	12	18
Tilia sp.	11	-	2	8	4	3	6	14	3	6	2	49	108
Tradescantia sp.	-	-	-	-	-	1	-	-	-	-	-	3	4
Trifolium pratense-Type	1	1	1	6	2	1	3	4	4	3	1	10	37
Trifolium repens-Type	10	-	2	8	3	3	6	14	4	6	2	46	104
Tropaeolum sp.	-	-	-	-	-	-	1	-	-	-	-	1	2
Ulmus sp.	1	-	-	1	-	-	-	-	2	-	-	2	6
unidentified_Munich-Type	-	-	-	-	-	-	-	4	-	-	-	-	4
unidentified_porate	-	-	-	-	-	-	-	-	-	-	-	2	2
unidentified_reticulate_tricolporate	-	-	-	2	-	-	-	1	-	2	1	14	20
unidentified_rugulate_tricolpate	2	-	-	3	-	-	2	3	-	-	1	1	12
unidentified_striate	-	-	-	-	-	1	-	1	-	-	-	1	3
unidentified_tetrad	-	-	-	1	-	-	-	-	-	-	-	-	1
unidentified_various	9	-	2	8	3	2	4	18	3	6	2	46	103

Pollen Type	**Bratislava (11)**	**Bregenz (1)**	**Graz (2)**	**Innsbruck (8)**	**Klagenfurt (4)**	**Linz (3)**	**Ljubljana (6)**	**Munich (17)**	**Prague (4)**	**Salzburg (6)**	**St. Poelten (2)**	**Vienna (50)**	**in n samples (of 114 total)**
unidentified_verrucate	-	-	-	1	-	-	-	-	-	-	-	-	1
Urtica sp./*Parietaria* sp.	4	-	1	3	2	3	2	10	1	1	-	24	51
Verbascum sp.	1	-	1	-	-	-	-	-	-	-	-	1	3
Veronica sp.	1	-	-	2	1	-	1	5	1	1	-	6	18
Viburnum lantana-Type	2	1	-	5	2	-	1	4	-	3	1	14	33
Viburnum sp.	1	-	-	4	1	1	3	7	-	1	-	12	30
Vicia sp.	3	-	-	-	-	-	1	5	1	-	-	4	14
Viola sp.	2	-	-	1	-	-	-	-	-	-	-	-	3
Viola tricolor	-	-	-	-	1	-	-	1	-	-	-	-	2
Viscum album	-	-	-	-	-	-	-	-	-	-	-	1	1
Vitis vinifera	3	-	-	3	-	-	1	1	-	-	-	16	24
Weigela sp.	1	-	-	-	-	-	-	3	-	-	-	-	4
Xanthium sp.	-	-	-	-	-	-	-	1	-	-	-	-	1
Yucca filamentosa	-	-	-	1	-	-	-	-	-	-	-	-	1
Zea mays/*Triticum aestivum*	-	-	-	5	2	-	6	6	2	1	1	5	28
Zelkova sp.	-	-	-	-	-	-	-	-	-	-	-	1	1
Zingiberaceae	-	-	-	-	-	-	-	1	-	-	-	-	1
Number of Samples per city	**11**	**1**	**2**	**8**	**4**	**3**	**6**	**17**	**4**	**6**	**2**	**50**	**114**

III. GRAPHICAL DESCRIPTION OF THE USED METHODS

a) Hydration (LM)

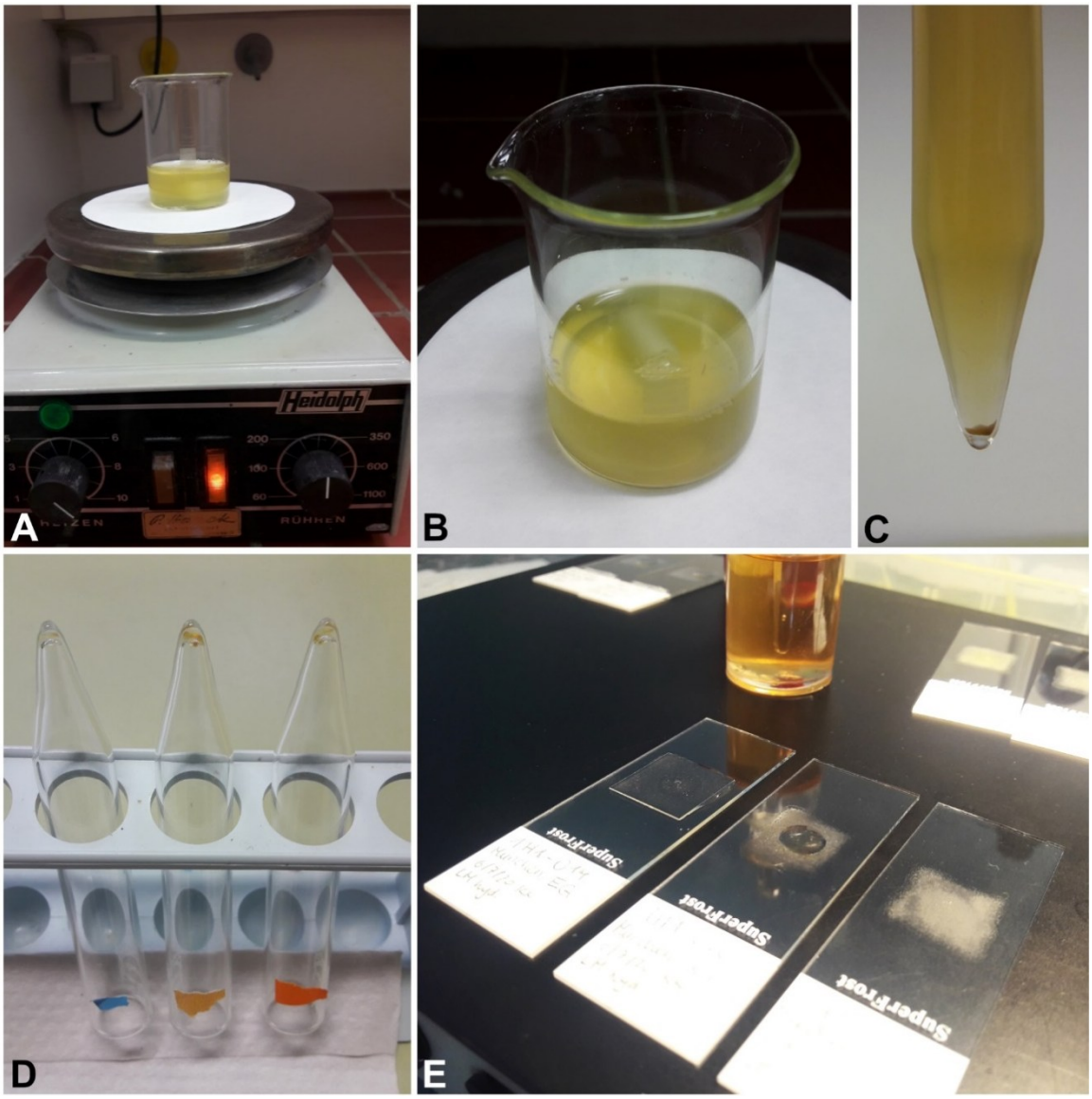


Figure A6: Preparing honey for light microscopy (hydrated pollen): Honey dissolves in water on a magnetic stirring machine (A–B), pollen pellet at the bottom of the glass tube after centrifuging (C), drying upside down glass tubes after decanting (D), pollen pellet is transferred onto a microscopic slide, embedded in glycerol gelatine, and covered with a cover slip (E).

b) Acetolysis (LM)

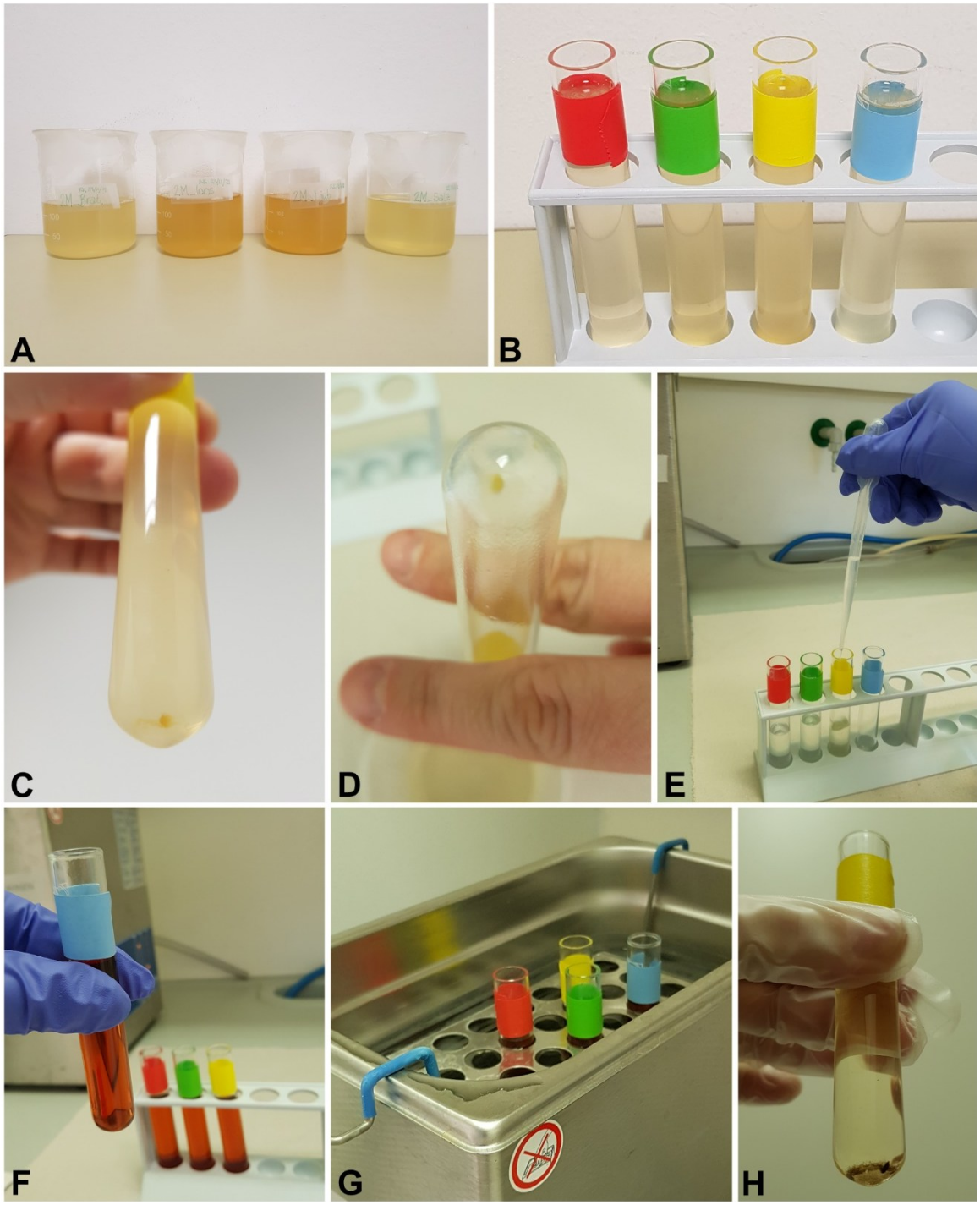


Figure A7: Acetolysis of honey samples, part I: Dissolving honey in water (A), multiple steps of centrifuging and decanting leads to pollen being concentrated at the bottom of the glass tube (B–D), adding acetic acid (E), samples with acetolysis mixture before (F), during (G), and after (H) heating the tubes in 80°C water for ten minutes.

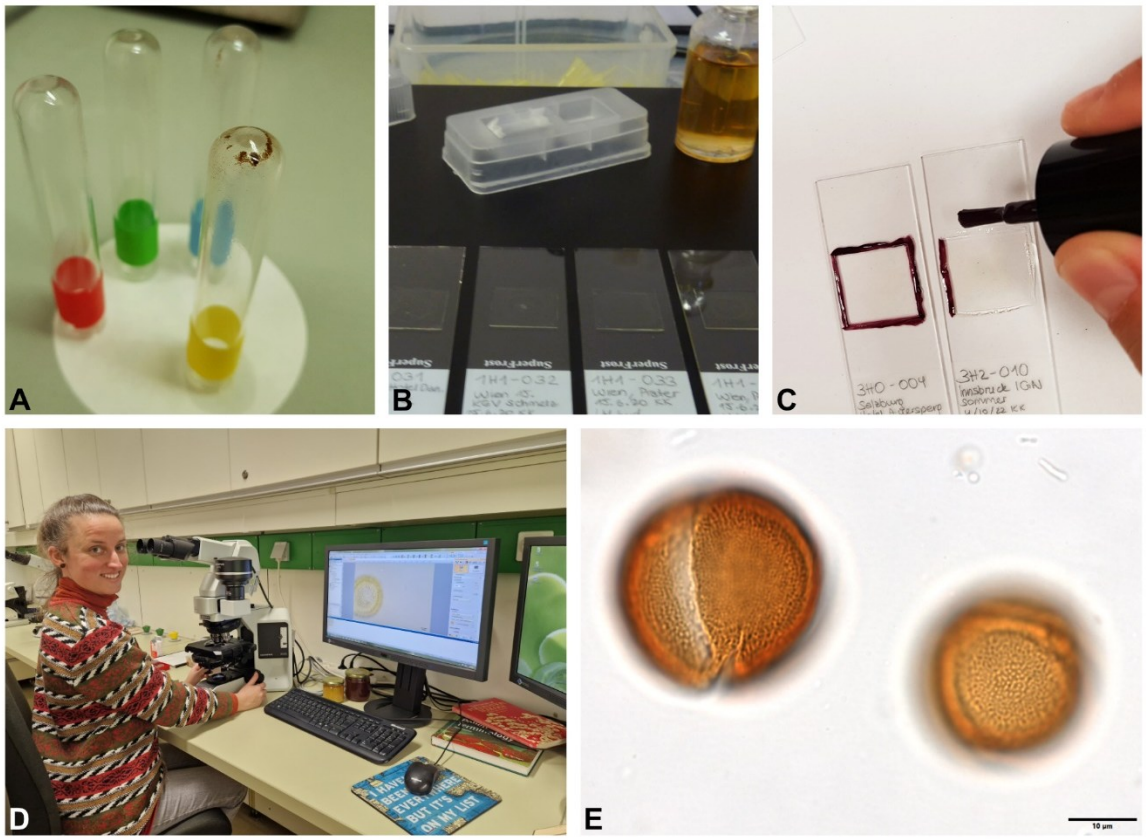


Figure A8: Acetolysis of honey samples, part II: After several washing steps the glass tubes with acetolysed pollen in water are placed upside down to dry (A), the pollen is transferred onto an object slide, embedded in glycerol gelatine with a cover slip (B), nail polish can be used to seal the sample (C), light microscopical analysis with x-1000 magnification (D), acetolysed pollen of *Acer* and *Rumex* (E).

c) SEM (acetolysed pollen)

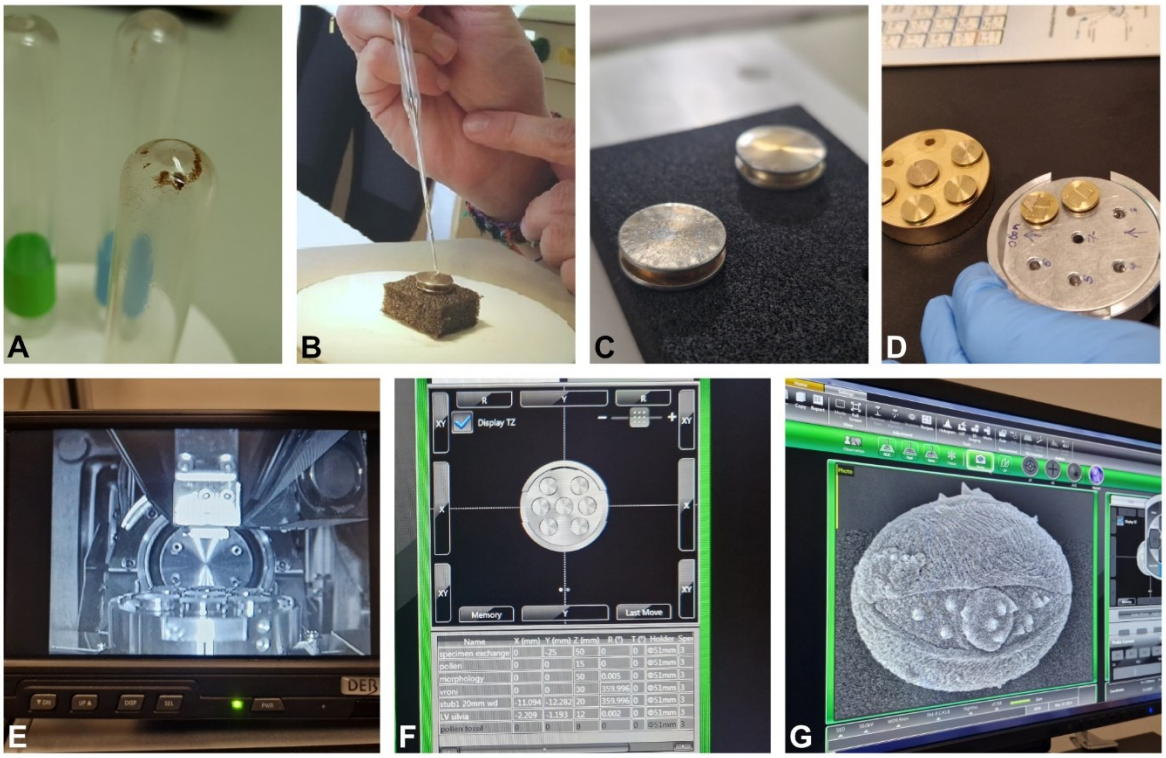


Figure A9: Preparing pollen from acetolysed honey for scanning electron microscopy (SEM): pollen sample after acetolysis (A), pollen in 96% ethanol is pipetted onto an aluminium pin stub (B), alcohol evaporates quickly (C), stubs are sputter-coated in gold and placed on the object holder of the scanning electron microscope (D), the object holder is carefully inserted into the specimen chamber (E), which is then vacuum sealed and the working distance of the electron beam is adjusted to the size of pollen grains (F) for photographic images to be generated on the computer (G).

d) LM reference slides

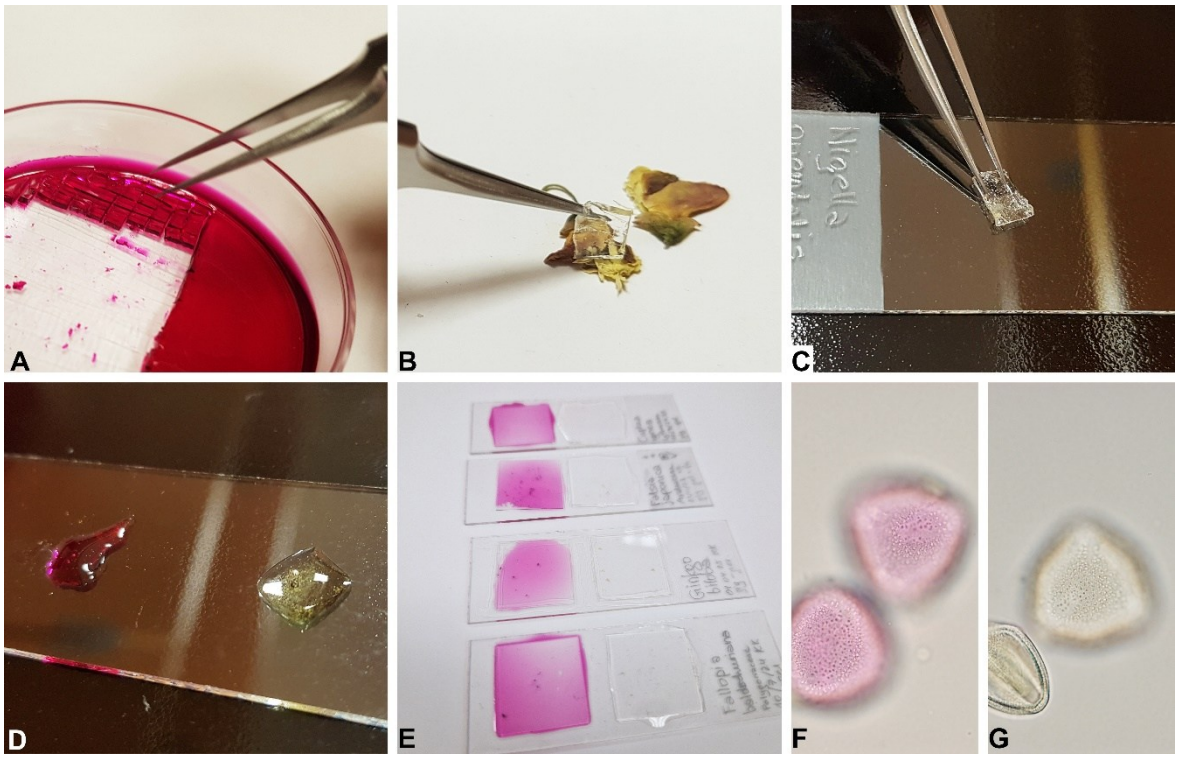


Figure A10: Preparing pollen of selected plant species for a new dataset on *PalDat*: glycerol gelatine (unstained and stained with basic fuchsin) is melted into a petri dish and, when hardened, cut in small cubes of approximately 0.5 x 0.5 x 0.1 cm (A), one cube is picked up with tweezers and pressed gently onto the anthers of a dried flower (B), the cube with attached pollen is transferred onto an object slide and melted on a heating plate (C–D), cover slips are added (E) and the specimen is analysed in the light microscope at x-1000 magnification, pollen of the respective plant species both stained (F) and unstained (G) is photographed and the pictures are prepared for upload following the instructions for authors on the *PalDat* website. A full list of prepared LM reference samples for *PalDat* can be found in the “List of Publications”.

IV. EXAMPLES OF POLLEN IDENTIFICATION WITH THE HELP OF ADDITIONAL METHODS AND REFERENCE

a) Identification with LM reference slides (hydrated)

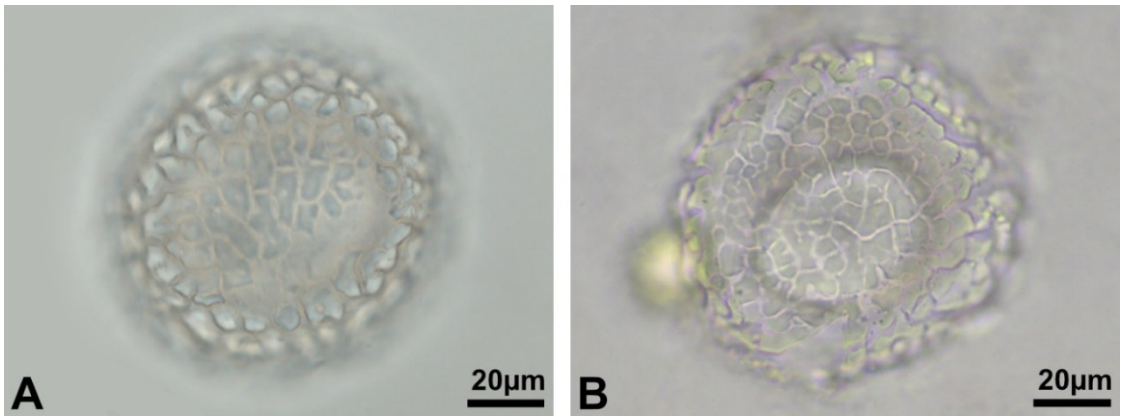


Figure A11: Unstained hydrated *Zingiber officinale* pollen grain prepared from fresh anthers for *PalDat* (A) and *Zingiber* sp. in a 2020 honey sample from Munich (B).

b) Identification with LM reference slides (acetolysed)

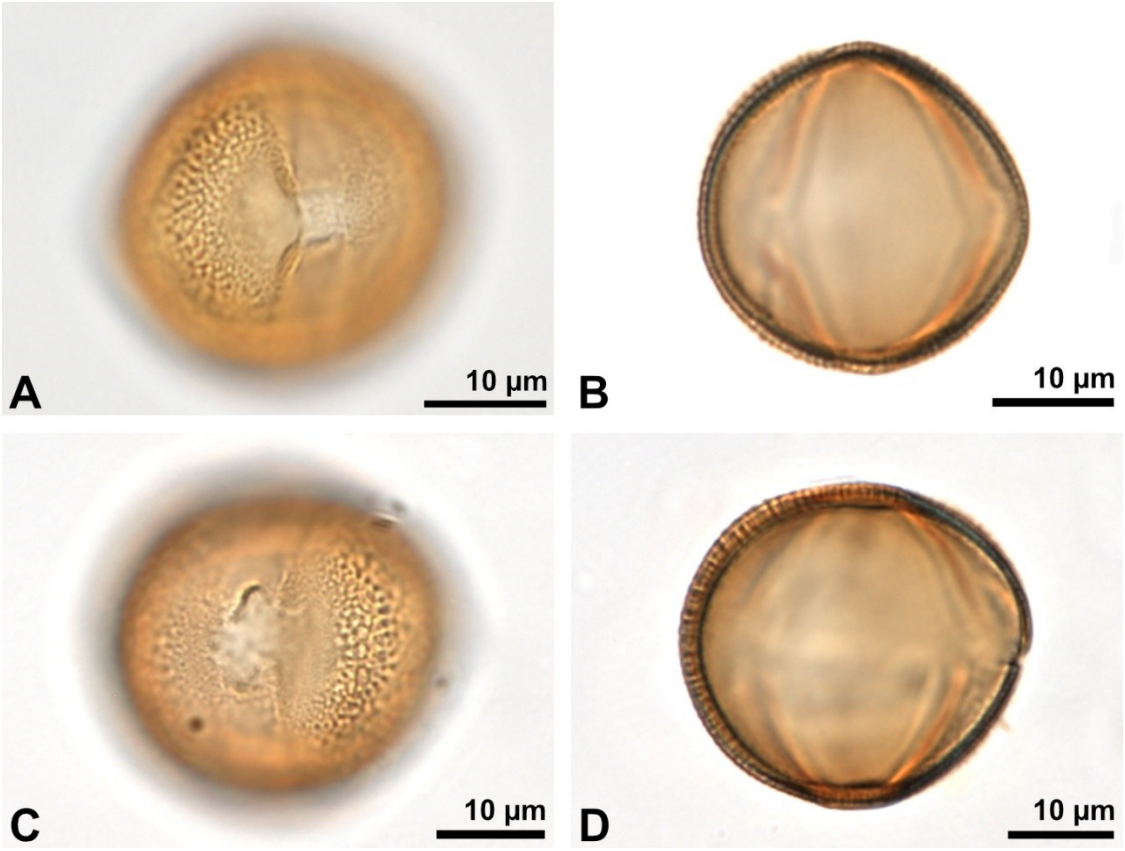


Figure A12: *Gleditsia triacanthos* pollen grain acetolysed from fresh anthers for *PalDat* (A–B) and *Gleditsia triacanthos* in an acetolysed 2020 honey sample from Innsbruck (C–D).

c) Distinguishing closely related plant taxa with the help of SEM

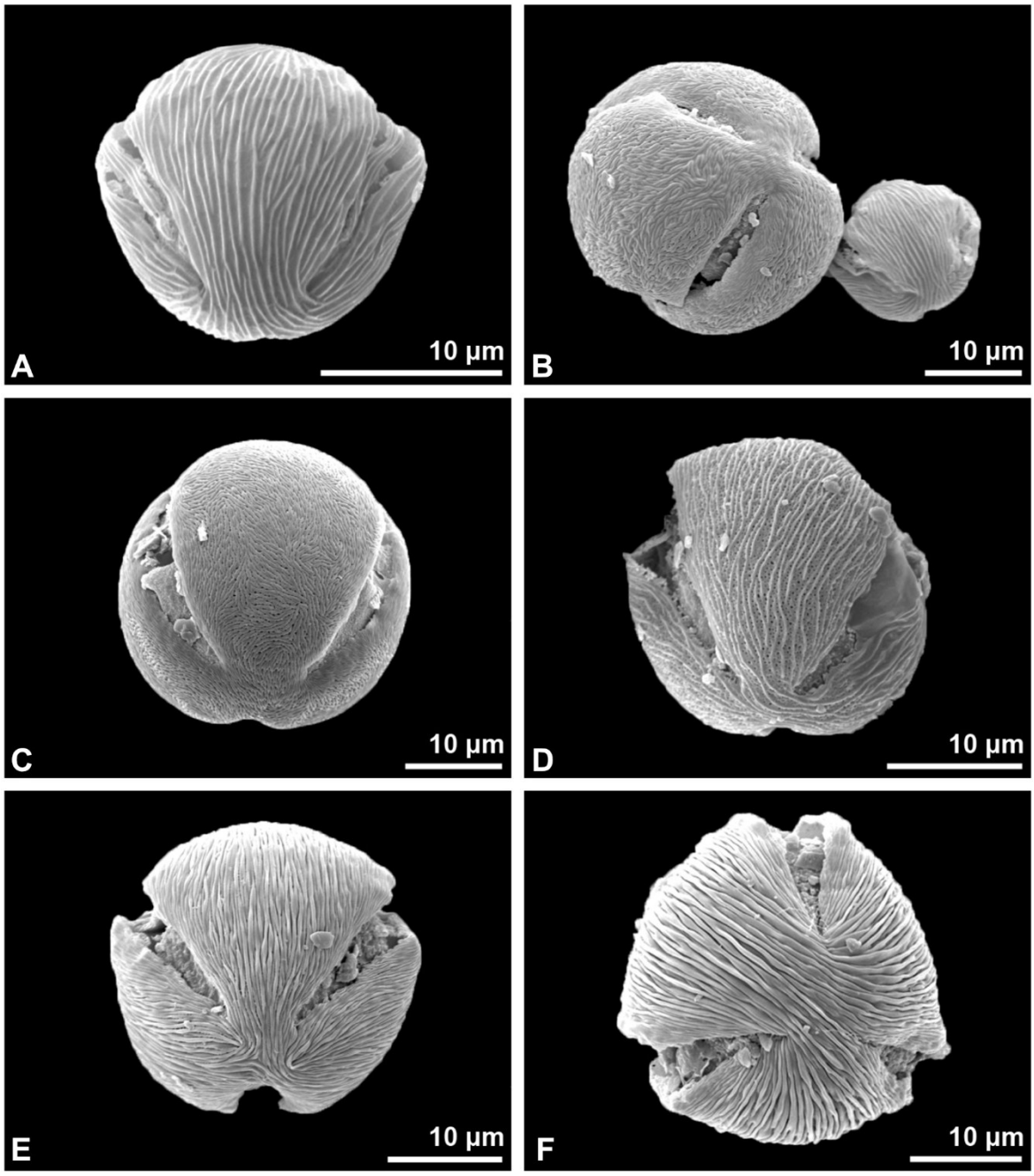


Figure A13: Pollen wall details of various Rosaceae species found in urban honey samples, acetolysed in SEM: *Fragaria* sp./*Potentilla* sp. (A), *Pyrus* sp. and *Fragaria* sp./*Potentilla* sp. (B), *Malus*-Type (C), *Rubus* sp. (D), and two different *Prunus* species (E–F).

d) Identification with the help of DNA-metabarcoding

DNA-metabarcoding of selected samples was conducted at the Institute for Food Safety, Austrian Agency for Health and Food Safety (AGES), Vienna. The nuclear ITS2 primer system was used in combination with the chloroplast *trnL* primers. The results allowed to relate a previously unidentified pollen grain from light microscopic analyses to the species *Sophora japonica*. The relative abundance of combined *Sophora japonica* reads as percentage of the total reads was 70.4% (Table A1), while the relative abundance of *Sophora japonica* pollen grains (Figure A14) in the LM analysis of the same sample was 63%.

Table A1: Dominance of *Sophora japonica* (syn.: *Styphnolobium japonicum*) according to the percentage of identified reads from sample 1H0-039.

Sample 1H0-039 (Vienna, 6, Hotal ibis Mariahilf)		
Taxonomy (Galaxy-Pipeline) customized Datenbank oder Datenbank (NCBI)	**NGS reads**	**% of reads**
…	…	…
Sophora japonica isolate PB032MT3	1	0.00
Sophora japonica voucher NCHU-D89431201-3003	73299	66.79
Styphnolobium japonicum isolate KPS0208A03	1	0.00
Styphnolobium japonicum voucher Duan 2016005 (IBSC)	1830	1.67
Styphnolobium japonicum voucher EDQM 32506	1009	0.92
Styphnolobium japonicum voucher EDQM 32963	9	0.01
Styphnolobium japonicum voucher M F Wojciechowski 856 (ASU)	3	0.00
Styphnolobium japonicum voucher P.Brownless 19698198 (E)	1049	0.96
Styphnolobium japonicum voucher Q636	61	0.06
…	…	…
Combined *Sophora japonica* reads	**77262**	**70.41**
Total Reads	**109739**	**100**

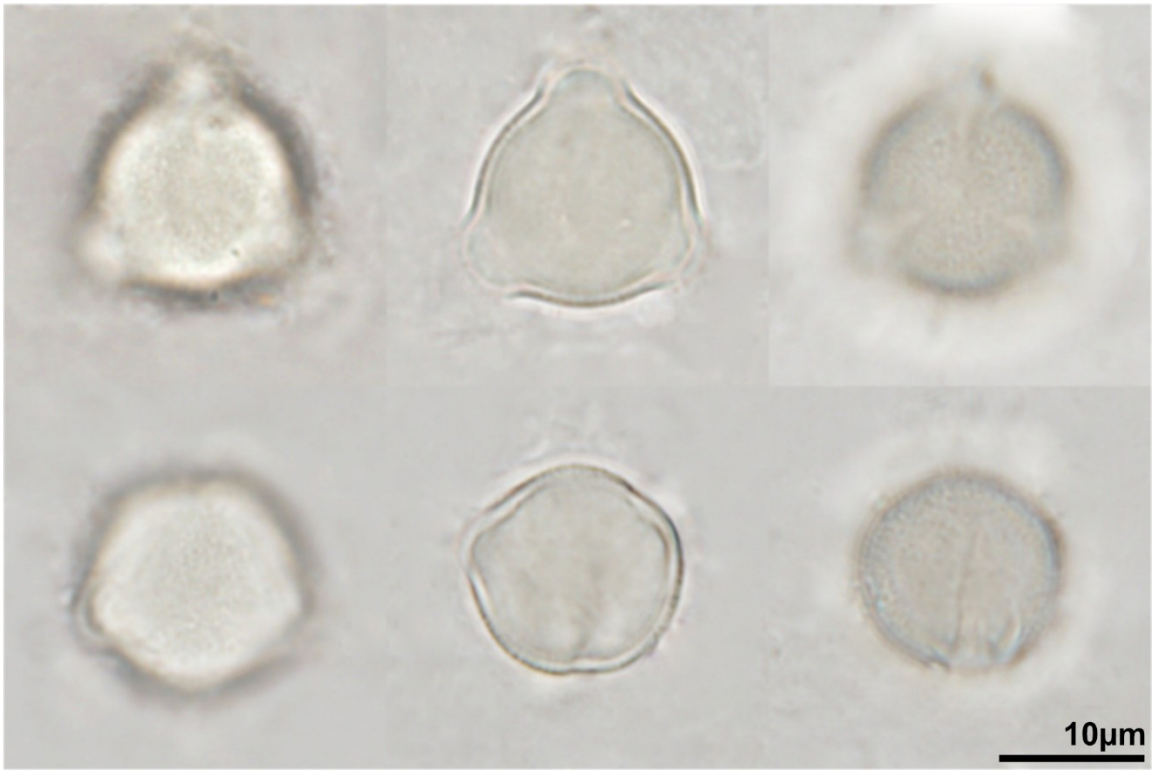


Figure A14: *Sophora japonica* pollen grain at x-1000 magnification in honey sample 1H0-039.