



universität
wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

“The *Hylaeus brevicornis* group revisited – an integrative approach to delimit four closely related species of masked bees (Hymenoptera: Apidae)”

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of
Master of Science (MSc)

Wien, 2018 / Vienna 2018

Studienkennzahl lt. Studienblatt /
degree programme code as it appears on
the student record sheet:

A 066 831

Studienrichtung lt. Studienblatt /
degree programme as it appears on
the student record sheet:

Masterstudium Zoologie

Betreut von / Supervisor:

ao. Univ.-Prof. Mag. Dr. Harald Krenn

Abstract

Hylaeus (masked bees) is a genus of solitary bees, consisting of approximately 47 species in Central Europe. Four morphologically similar species of the subgenus *Dentigera*, *Hylaeus brevicornis* NYLANDER, 1852, *Hylaeus gredleri* FÖRSTER, 1871, *Hylaeus intermedius* FÖRSTER, 1871, and *Hylaeus imparilis* FÖRSTER, 1871, were studied. To clarify their still ambiguous relatedness, pollen analyses, as well as molecular and morphometric analyses were carried out in order to find potential interspecific differences. Most specimens were collected on the area of the former Nordbahnhof in Vienna, where the species occur syntopically.

As females of *Hylaeus* do not have an exterior scopa, but the pollen is swallowed and transported in a crop, the metasoma was dissected in order to study the crop content. Pollen analyses showed that the four investigated *Hylaeus* species are generally polylectic, collecting pollen of different plant families. They do have preferences for plants with small florescences like Apiaceae, Brassicaceae and Crassulaceae, depending on their availability. Between the species no major differences in pollen composition of the crop content were detected.

Results gained from DNA barcoding largely agree with previous studies and support morphological determinations. While molecular data confirm the monophyly of each of the three species *H. brevicornis*, *H. imparilis* and *H. gredleri*, "*H. intermedius*" in the present sense split up in two distinct clades. Differences between the investigated species and the two clades of *H. intermedius* were also discovered by morphometric measurements of head structures. Large intraspecific size variations, though, complicate a reliable determination of females on basis of morphology. This problem should be revisited in the light of the arisen two-species-hypothesis of *H. intermedius*.

Zusammenfassung

Hylaeus (Maskenbienen) ist eine Wildbienengattung, die in Mitteleuropa aus ungefähr 47 Arten besteht. Untersucht wurden vier morphologisch ähnliche Arten der Untergattung *Dentigera*, *Hylaeus brevicornis* NYLANDER, 1852, *Hylaeus gredleri* FÖRSTER, 1871, *Hylaeus intermedius* FÖRSTER, 1871, und *Hylaeus imparilis* FÖRSTER, 1871. Um ihre immer noch ungewissen Verwandtschaftsverhältnisse zu klären und etwaige zwischenartliche Unterschiede zu finden, wurden sowohl Pollenanalysen, als auch molekulare und morphometrische Analysen durchgeführt. Die Exemplare wurden größtenteils auf dem ehemaligen Nordbahnhofareal in Wien gesammelt, wo die Arten syntop vorkommen.

Weil *Hylaeus*-Weibchen keine äußeren Sammeleinrichtungen besitzen, sondern der Pollen geschluckt und in einem Kropf transportiert wird, wurde das Metasoma präpariert, um den Kropfinhalt zu analysieren. Pollenanalysen zeigten, dass die vier untersuchten *Hylaeus*-Arten

generell polilektisch sind und Pollen von unterschiedlichen Pflanzenfamilien sammeln. Sie haben aber eine Vorliebe für kleinblütige Pflanzen, wie Apiaceae, Brassicaceae oder Crassulaceae, abhängig von deren Verfügbarkeit. Zwischen den Arten wurden keine wesentlichen Unterschiede in der Pollenzusammensetzung des Kropfinhaltes gefunden.

Ergebnisse, die mittels DNA-Barcoding gewonnen wurden, stimmen größtenteils mit vorhergehenden Studien überein und unterstützen die Bestimmung auf morphologischer Basis. Während die molekularen Daten die Monophylie der drei Arten *H. brevicornis*, *H. imparilis* und *H. gredleri* bestätigen, stellte sich heraus, dass sich die Art „*H. intermedius*“ im herkömmlichen Sinn in zwei verschiedene Clades aufspaltet. Unterschiede zwischen den untersuchten Arten und den beiden Clades von *H. intermedius* wurden auch durch morphometrische Messungen von Kopfstrukturen festgestellt. Beträchtliche intraspezifische Größendifferenzen erschwerten aber eine zuverlässige Bestimmung der Weibchen auf Basis von Morphologie. Dieses Problem soll angesichts der entstandenen Zwei-Arten-Hypothese von *H. intermedius* erneut aufgegriffen werden.

Acknowledgements

Sincere thanks are owed to colleagues from Natural History Museum Vienna, Herbert Zettel and Dominique Zimmermann for their great overall support and expertise. I especially like to thank Dominique Zimmermann for providing collected *Hylaeus* specimens from the project “Genetische Vielfalt der Wildbienen Österreichs” and Herbert Zettel for providing specimens from his private collection. Furthermore, I am grateful to supervisor Harald Krenn and the Department of Integrative Zoology (University of Vienna) for providing required material and funding parts of DNA barcoding. I also thank Harald Letsch, Brigitte Gottsberger (both from University of Vienna) and Lukas Zangl (Karl-Franzens University, Graz) for their support with DNA barcoding and DNA data processing. I want to express my gratitude to Alice Laciny (Natural History Museum Vienna) for her introduction into the methods of morphometry and statistical evaluation and to Sophie Kratschmer (Universität für Bodenkultur Wien) for explaining how to generate and evaluate pollen samples. Finally, I thank Göran Holmström from Sweden for sharing his expertise about masked bees and Angelika Schoder for supporting with verbal skills and proofreading.

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1. Introduction

Bees (Apidae s.l., or Apiformes) vary a lot in behavior as well as appearance, including a wide range from very small to large individuals (WESTRICH 1989). Only in Austria, 696 bee species occur (WIESBAUER 2017). *Hylaeus* – colloquially called “masked bees” due to their white to yellow face masks – is a genus of solitary bees consisting of approximately 47 species in Central Europe (DATHE et al. 2016). Masked bees are distributed on all continents except Antarctica, representing 650 species worldwide, with the highest species diversity in temperate and subtropical Australia (SCHEUCHL & WILLNER 2016).

The focus of my thesis is on *Hylaeus* species of the subgenus *Dentigera*, which can be divided in two groups: the *brevicornis* group and the *conformis* group (DATHE 1980). ALFKEN (1904) merged several species from the subgenus *Dentigera* to one polytypic species “*H. brevicornis*”. Although this was criticized various times, the first to revise it was DATHE (1980). He distinguished at least seven discrete species in the *Hylaeus brevicornis* group: *H. brevicornis* NYLANDER, 1852, *H. gredleri* FÖRSTER, 1871, *H. imparilis* FÖRSTER, 1871, *H. kahri* FÖRSTER, 1871, *H. punctus* FÖRSTER, 1871, *H. glacialis* MORAWITZ, 1872, and *H. breviceps* MORAWITZ, 1876 (DATHE 1980). Recently, one more species, *H. intermedius* FÖRSTER, 1871, was reintroduced based on morphology (DATHE et al. 2016), which was formerly regarded as a synonym of *H. gredleri*. Most species of the *Hylaeus brevicornis* group are challenging to distinguish by morphology alone, and DNA analyses are advisable for a clear determination (DATHE et al. 2016). Generally, integrative approaches – the use of complimentary disciplines for solving taxonomic problems – are useful if morphological methods fail (SCHLICK-STEINER et al. 2010). The clearest result for solving a taxonomic problem is agreement among various used approaches (SCHLICK-STEINER et al. 2010).

Pollen – combined with nectar, or in rare cases oil – represents an essential part of larval food for all non-parasitic bee species (WESTRICH & SCHMIDT 1986, WESTRICH 1989). The pollen is collected by female bees in different ways. Many species, like the well-known honey bee (*Apis mellifera*), have particular appliances for carrying the pollen on their legs. Others, mostly in the subfamily Megachilinae, carry the pollen with the help of an abdominal scopa (WESTRICH 1989). *Hylaeus* does not have an exterior scopa, but the pollen is taken with the help of their front tarsi as well as special bristle combs on the galea of the maxilla and swallowed. It is stored and transported to the nest in a crop, which is located in the front part of the metasoma. Arriving at the nests, a mixture of pollen and nectar is regurgitated and stored in the brood cells (JANVIER 2012).

Bees also vary in their preference for certain plants from which they gather pollen for their offspring. Whereas species which collect pollen from only one or several closely related plant species are defined as oligolectic, species which use a variety of unrelated plants as pollen

source are called polylectic. However, this does not refer to visiting other plants as nectar source for their own needs (WESTRICH 1989). Furthermore, the terms oligolectic and polylectic have to be distinguished from flower steadiness. The latter describes the phenomenon that one bee individual may prefer a single flower species during one flight for collecting pollen. This is also common within polylectic bee species (WESTRICH & SCHMIDT 1986).

Whereas for other bee species many pollen analyses have been carried out yet (e.g. WESTRICH 2008, MÜLLER & KUHLMANN 2008, and WESTRICH 2016), very few studies are available in case of the genus *Hylaeus* (e.g. WILSON et al. 2010). As the analysis of the crop content requires time consuming dissections in this genus, data about pollen sources was mainly gained by observations so far, which are not very reliable as bees also visit flowers for drinking nectar. Most Central European *Hylaeus* species are supposed to be generalist pollen foragers, except of four species: *H. signatus* (PANZER, 1798) collects on *Reseda* only, *H. nigrinus* (FABRICIUS, 1798) on Asteraceae and *H. punctulatissimus* SMITH, 1842 as well as *H. bifasciatus* (JURINE, 1807) collect on *Allium* (WESTRICH 1989, SCHEUCHL & WILLNER 2016). However, a recent study of the crop content provided evidence that *H. punctulatissimus* is not strictly oligolectic, but can also use other pollen sources (SCHODER & WIESBAUER 2017). In WESTRICH (1989) the species *H. brevicornis* is described to be polylectic and observed on Apiaceae (*Heracleum*, *Daucus*), Campanulaceae (*Jasione*), Crassulaceae (*Sedum*) and Rosaceae (*Rubus*). In that study, *H. gredleri* is treated as a synonym of *H. brevicornis* and other species from the *Hylaeus brevicornis* group are not mentioned as they do not occur in the study area.

My thesis is designed to fill some gaps in the knowledge of the *Hylaeus* subgenus *Dentigera*, specifically the *Hylaeus brevicornis* group.

Sympatric occurrence of four species from the *Hylaeus brevicornis* group on the premises of the former Nordbahnhof in Vienna – *H. brevicornis*, *H. gredleri*, *H. intermedius* and *H. imparilis* – represented a unique opportunity to explore differences in feeding preferences between them. By analyzing the crop content, I aim at investigating whether the four selected *Hylaeus* species are oligolectic or polylectic, whether they show flower steadiness, and whether they differ in their feeding preferences in the study area. Furthermore, their most important pollen sources shall be identified.

DNA barcodes enable a more reliable species determination, and possible irregularities within the newly established species *H. intermedius* might be detected. Additionally, morphometric analysis of head structures shall track potentially measureable differences between the investigated species.

A final synthesis of the results shall clarify if there are ecological, genetic or morphological differences between *Hylaeus brevicornis*, *H. imparilis*, *H. gredleri* and *H. intermedius*.

2. Material and methods

2.1. Material collection

Most of the investigated *Hylaeus* specimens were caught from June 2016 to June 2017 on a small area in Vienna, the premises of the former Nordbahnhof. In summer 2016 *Hylaeus* species for the thesis were mainly collected together with other solitary bee species in the course of the project “Genetische Vielfalt der Wildbienen Österreichs” by the Natural History Museum Vienna. As determination in the field was not possible, all *Hylaeus* species were taken. The bees were caught with an insect net and killed in tubes with ethyl acetate vapor or ethanol. The specimens of the four selected species (*H. brevicornis*, *H. gredleri*, *H. intermedius*, and *H. imparilis*) were sorted out and represented only a small amount of the total number of *Hylaeus* specimens collected by the author. Whereas some females were pin-mounted, most of them were stored in tubes with 96% ethanol. Of each individual one hind leg was cut off for genetic analysis and stored separately in 96% ethanol in a freezer to maintain the DNA.

For morphometric measurements as well as molecular analyses, additional specimens of the subgenus *Dentigera* were loaned from the private collection of Herbert Zettel. Many of those were also caught on the premises of the former Nordbahnhof, but some originated from Lower Austria or other areas in Vienna. This became especially necessary in case of *H. imparilis* and *H. gredleri* because not enough individuals of these two species could be collected by the author herself. Also, a gynander of *H. intermedius*, published by SCHODER & ZETTEL (2017), was used for this study. For morphometric measurements one more species, *H. kahri*, was added, collected on Perchtoldsdorfer Heide, in the periphery of Vienna by H. Zettel. Furthermore, measurements were carried out on the female syntype of *H. gredleri*, loaned from the “Zoologische Staatssammlung München” (ZSM).

All investigated specimens are listed in table Appendix 1.

2.2. Study area

Fieldwork was carried out on the premises of the former Nordbahnhof in the 2nd district of Vienna (Austria), approximately 500 m away from the Danube River (Fig. 1, N48°13'33–50", E16°23'24–38").

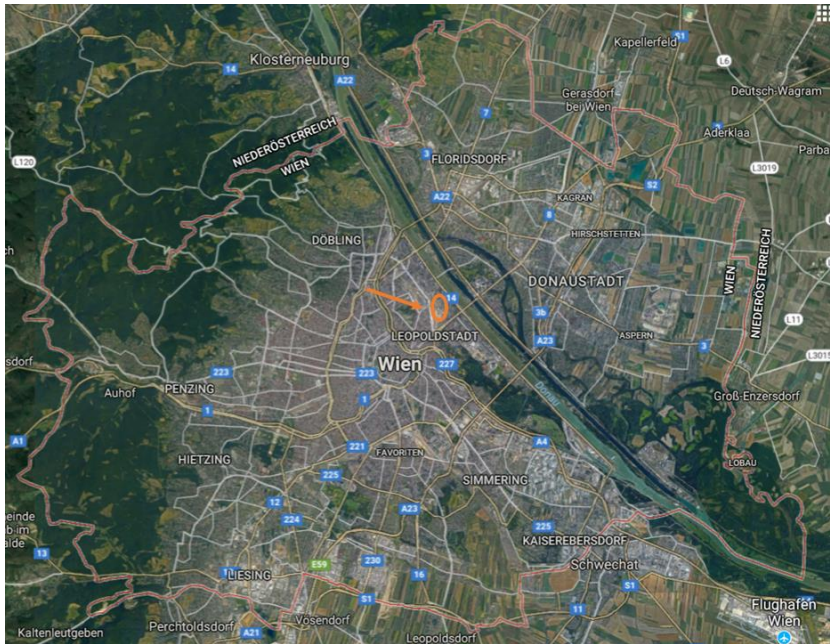


Fig. 1. Map of Vienna; with the premises of the former Nordbahnhof inside the orange circle (Map based on Google maps and edited by S. Schoder).

Up to World War II, the so-called “Nordbahnhof” was of great importance and splendor, but it got heavily destroyed during the war and completely demolished in 1965. Since the 1980s the premises of the former Nordbahnhof began to be refurbished into another urban district (VLAY et al. 2014). With a total area of 85 hectares, it was one of the most important urban zones of development.

At approximately half of the Nordbahnhof area construction is already finished (VLAY et al. 2014). The other half still represents a large industrial wasteland centrally located in Vienna, characterized by artificially raised railroad embankments, several retaining walls, tunnels and vegetated banks. The whole area can be described as an extremely dry habitat with very high surface temperatures in summer (SCHINNINGER et al. 2002).

Beside the high flower diversity, the premises of the former Nordbahnhof offer good breeding conditions for solitary bees, resulting in a diverse bee fauna. Therefore the Nordbahnhof is one of the current bee hotspots in Vienna including approximately 180 bee species and 22 species of *Hylaeus*, collected in 2016 and 2017 (ZETTEL et al. unpubl.).

In the coming years until 2025, the remaining Nordbahnhof area is planned to be built up. In the concept of 2014, though, a free middle area and green space of approximately 12 hectares is planned, enabling people to experience and use nature (VLAY et al. 2014).

For collecting Hymenoptera, the area of the former Nordbahnhof was subdivided into ten sampling sites (Fig. 2). However, the specimens used for pollen analyses were only sampled on four of these sites: Nbh1 (N48°13'34", E16°23'37"), Nbh2 (N48°13'40", E16°23'30"), Nbh3 (N48°13'47", E16°23'28"), and Nbh4 (N48°13'45", E16°23'23"). The coordinates

represent the center of each sampling site. Furthermore the site Nbhf3 includes the area of Nbhf8 in this study (Fig. 2).



Fig. 2. The premises of the former Nordbahnhof subdivided into 10 sampling sites. The horizontal line shows the border between the two grid squares L13 and M13 in the Freytag & Berndt city map used as a base for the bee inventory project (Map based on Google Earth and edited by H. Zettel).

Nbhf1 was characterized mainly by sand and gravel as well as some green along the edges involving a variety of flowering plants (Fig. 3a). Nbhf2 contained several retaining walls of red-brick, embankments and tunnels, probably ideal breeding conditions for bees and other hymenoptera. Plants were scattered all around mainly on sandy ground. (Fig. 3b). However, the sites Nbhf1 and Nbhf2 are already under construction since spring 2017. Site Nbhf3 is overgrown with a high diversity of flowering plants and crossed by several gravel paths. Nbhf3 merges into Nbhf8, which is also heavily overgrown, but with a sandier ground, a broad gravel path, and more trees (Fig. 3c). Nbhf4 is characterized by shrubs, perennials and sandy as well as green areas crossed by partly overgrown rails. Furthermore, this site contains the former gateman's house (Fig. 3d).



Fig. 3a–d. The sampling sites on the premises of the former Nordbahnhof: Nbhf1 (a), Nbhf2 (b), Nbhf3 (c) and Nbhf4 (d).

2.3. DNA barcoding

Genetic analyses were partly carried out by the author herself in a practical course in the study programme Zoology at the University of Vienna. Analyses of some other specimens were outsourced, partly to the Institute for Zoology (Karl-Franzens-University, Graz), and partly to AIM GmbH (Munich).

During the practical course DNA barcodes of 21 samples could be generated successfully. DNA extraction was carried out with the help of a peqlab Kit (peqGOLD Tissue DNA Mini Kit), using one leg and parts of the metasoma of each specimen. By means of polymerase chain reaction (PCR) the desired *COI* (Cytochrome c oxidase subunit I) gene was amplified in the PCR machine “Mastercycler” (Eppendorf), using the LCO-HCO primer pair. Gel electrophoreses should clarify if the amplification worked. After removing the surplus material (primer and nucleotides), the amplified *COI* genes were sequenced in the “3730 Genetic Analyzer” (ThermoFisher Scientific). The crude generated sequences were processed with help of the program SegMan Pro version 7.1.0 and aligned with BioEdit version 7.2.3.

Of the 50 samples sent to the Karl-Franzens-University of Graz, 26 DNA barcodes could be generated. DNA extraction was done by a different method there, using the solutions Quanta

BioScience Extraction Reagent and Quanta BioScience Stabilization Buffer. The tissue of each sample was smashed directly in Quanta BioScience Extraction Reagent and then cooked for 30 minutes. After cooking, Quanta BioScience Stabilization Buffer was added to carry on with DNA amplification, also by means of the LCO-HCO primer pair.

Five of the unsuccessful samples were again sequenced at AIM GmbH in Munich using the LepF-LepR primer pair. DNA barcodes could be generated there in four of the five specimens.

The received *COI* sequences were matched to reference data in BOLD (Barcode of Life Database version 4, RATNASINGHAM & HERBERT 2007) in order to detect highest similarities and therefore facilitating determination. Together with data from BOLD a Neighbor Joining tree including Bootstrap values was generated with the program Mega version 7.0.25 (KUMAR et al. 2016), as well as a Maximum Likelihood tree including Bootstrap values with IQ-tree version 1.5.5. Both phylogenetic trees were illustrated in FigTree version 1.4.3.

2.4. Pollen analyses

For generating pollen samples the metasoma was cut off and dissected, so that the pollen could be removed from the crop. It turned out that metasomas which were dried for approximately half an hour after extraction from ethanol are the easiest to dissect, whereas a completely dried interior crumbles, and a wet one is rather sticky and therefore difficult to handle. The extracted pollen was placed on microscope slides and crushed with a spatula. Afterwards it was intermixed with a solution of Kaiser's glycerol gelatin and some drops of fuchsine for dyeing. The stain enables a better distinction of the pollen grains. However, too much coloration is unfavorable as it prevents a proper examination of pollen structure. The solution of glycerol gelatin and fuchsine was heated in a bain-marie so that it became liquid. On the microscope slides it cools down and hardens again. This technique enables producing permanent pollen samples.

Pollen analyses were performed under an Olympus CX41RF-5 light microscope. Determination was carried out with help of literature (BEUG 2004, HESSE et al. 2009), databases (PalDat¹ and pollen.tstebler²), as well as reference flowers from the study site in case of uncertainty. Terminology follows HESSE et al. (2009). To recognize all important structures of the pollen grain it was necessary to focus through the different layers, in order to see either the surface or the inner structure (Fig. 4). A list of flowers occurring at the sampling

¹ PalDat – a palinological database (2000 onward, <https://www.paladat.org/>), May 15, 2017

² <http://pollen.tstebler.ch>, May 15, 2017

area – determined with help of literature (ADLER et al. 1994, SPOHN et al. 2008) – was generated by the author to facilitate pollen determination (Appendix 2).

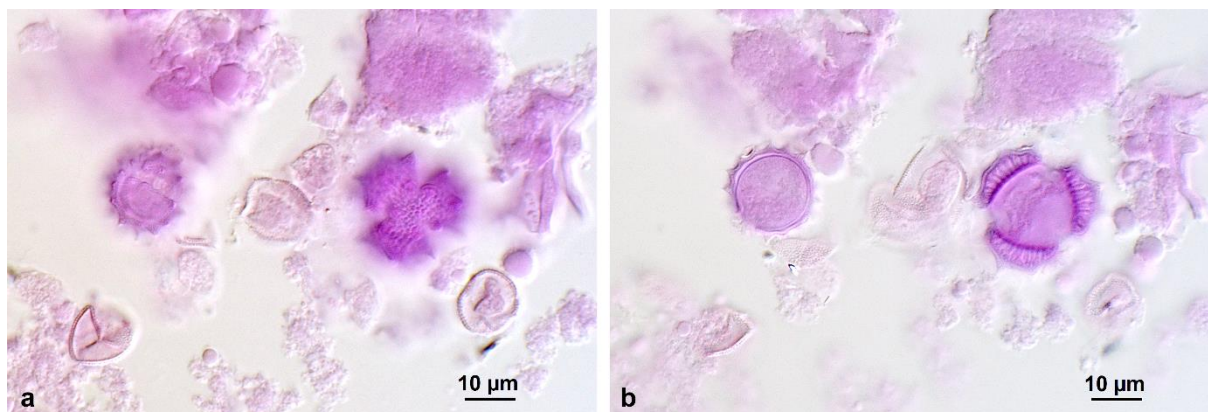


Fig. 4. Focus layers of the pollen grains. a. Focus layer 1, showing the surface structure of the pollen grains. b. Focus layer 2, showing the mid-section of the pollen grains (Photos taken with a Nikon Eclipse E800 microscope).

The determined pollen grains were grouped into the following types or families: Apiaceae, Brassicaceae, Asteraceae tubuliflorae, Asteraceae liguliflorae, Caryophyllaceae, Triangular/tricolporate and Undetermined (Fig. 5a–f). Apiaceae pollen is rather homogenous, tricolporate, prolate to rod-shaped and psilate (Fig. 5a). Brassicaceae pollen is tricolpate, spheroidal to prolate and recognizable by an intense reticulum (Fig. 5b). Whereas pollen of the type Asteraceae tubuliflorae is spheroidal, tricolporate and echinate, pollen of the type Asteraceae liguliflorae is spheroidal, tricolporate and fenestrate (Fig. 5c, d). By means of light microscopy no reliable distinction was possible between the families Rosaceae and Crassulaceae and the genus *Linaria* of the Plantaginaceae, therefore they were all placed in one common group: Triangular/tricolporate. In this case the pollen grains are triangular to spheroidal, tricolporate and reticulate to striato-reticulate (Fig. 5e). Caryophyllaceae pollen is again easy to determine, as it is spheroidal and pantoporate, containing a high number of pores all over the surface (Fig. 5f).

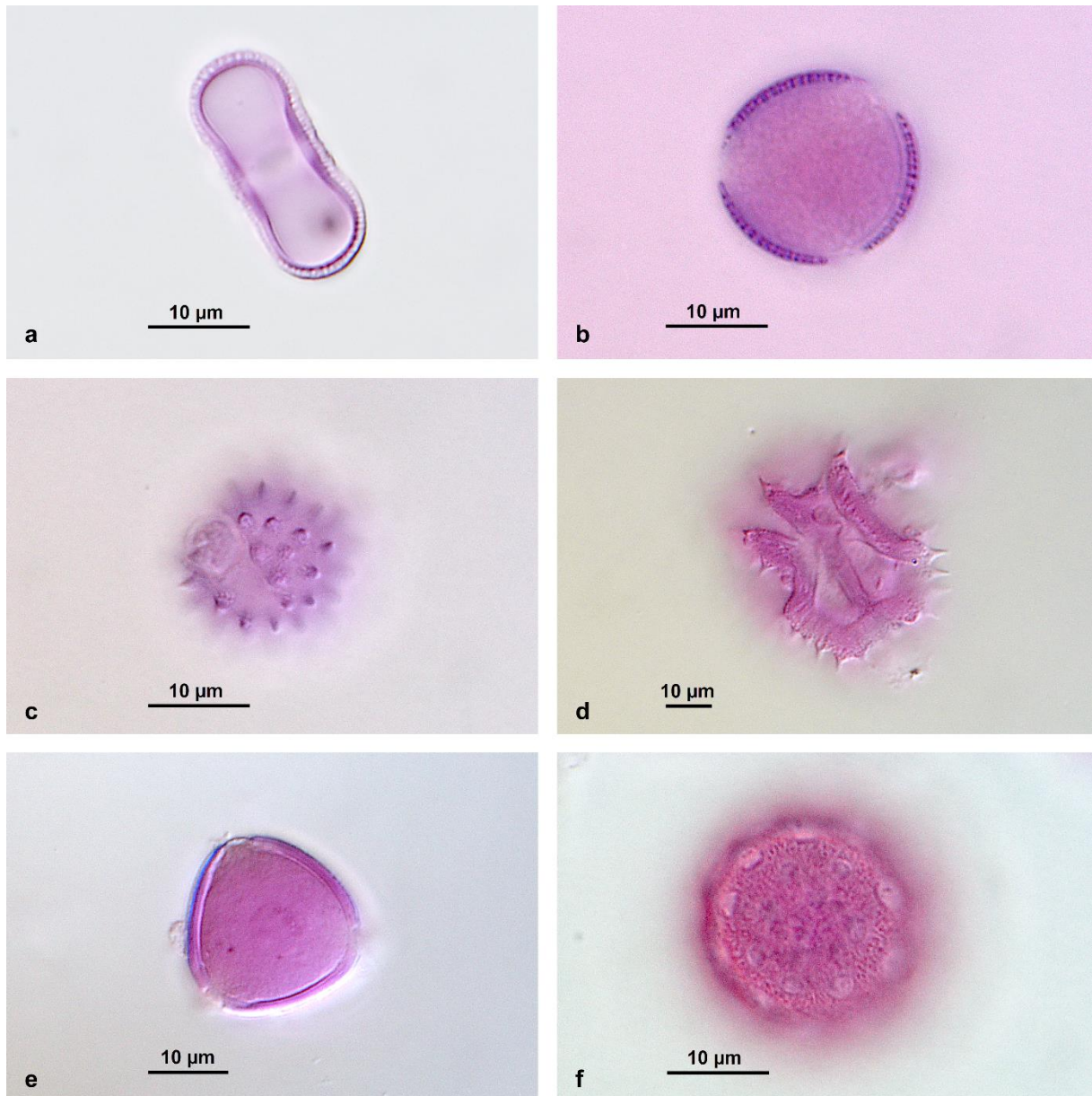


Fig. 5. One representative of each pollen type or family. a. Apiaceae; b. Brassicaceae; c. Asteraceae/tubuliflorae; d. Asteraceae/liguliflorae; e. Triangular/tricolporate; f. Caryophyllaceae (Photos taken with a Nikon Eclipse E800 microscope).

Approximately 300 pollen grains per sample were screened (using the Olympus CX41RF-5 microscope) and each grain was noted at the respective family or type. Percentages of pollen composition per individual and species, as well as mean values were calculated and illustrated with Microsoft Excel 2013.

2.5. Morphometric measurements

Morphometric measurements were restricted to structures of the head capsule and carried out for all *Hylaeus* females used for pollen analyses plus female specimens from the collection of H. Zettel and one *H. gredleri* syntype from the “Zoologische Staatssammlung München” (ZSM). Complimentary to determination based on morphology, DNA barcodes were generated in 40 out of the 70 measured individuals for a pre-sorting of the specimens. Altogether, the heads of 18 *H. brevicornis*, 21 *H. gredleri*, 15 *H. imparilis*, 22 *H. intermedius* (including the gynandromorph specimen), and two *H. kahri* specimens were measured. Three female specimens that were morphologically identified as *H. intermedius* but cluster outside the rest of this species in the DNA-based phylogenetic tree were labeled as *H. intermedius* group 2.

The following measuring lengths were used (Fig. 6):

Head length (HL). Maximum length of head in full-face view, excluding mandibles, measured from anterior-most point of clypeus to posterior point of head vertex, parallel to midline.

Head width (HW). Maximum width of head in full-face view (including eyes).

Clypeus length (CL). Maximum length of clypeus in full-face view, measured from anterior-most point to posterior-most point of clypeus, parallel to midline.

Clypeus width (CW). Width of clypeus measured basally, at the border to the subantennal sclerite.

Clypeus–antenna distance (CA). Length from one posterbasal corner of clypeus to the nearest point of antennal fossa, including elevated ridge.

Eye width (EW). Maximum width of compound eye in right angle to eye axis, measured in exact lateral view of head.

Temple width (TW). Maximum width of temple, measured in exact lateral view of head.

Head depth (HD). Maximum width of head in lateral view; sum of eye width and temple width.

In addition to the absolute values, relative values in the form of indices were used:

Cephalic index. $HW / HL \times 100$

Clypeus index. $CW / CL \times 100$

Head depth index. $HD / HL \times 100$

Temple width index. $TW / EW \times 100$

CA index. $CA / CW \times 100$

Most measurements were taken by means of an Olympus SZH10 binocular microscope at a magnification of 60×, but CA and CW were additionally measured with a Nikon SMZ1500 binocular microscope at a magnification of 78×. The values obtained from the calibrated ocular micrometres were converted to millimetres.

Mean values (M), as well as minimum (min) and maximum (max) values of each measurement and index were calculated with Microsoft Excel 2013 for each species, except for *H. kahri* and *H. intermedius* group 2, as not enough specimens of these two clades were studied. Relevant ratios of all investigated specimens were illustrated as scatter diagrams.

Furthermore, a principal component analysis based on a variance-covariance matrix of log-transformed data was carried out for all measured parameters with the program PAST version 3.17 (HAMMER et al. 2001)

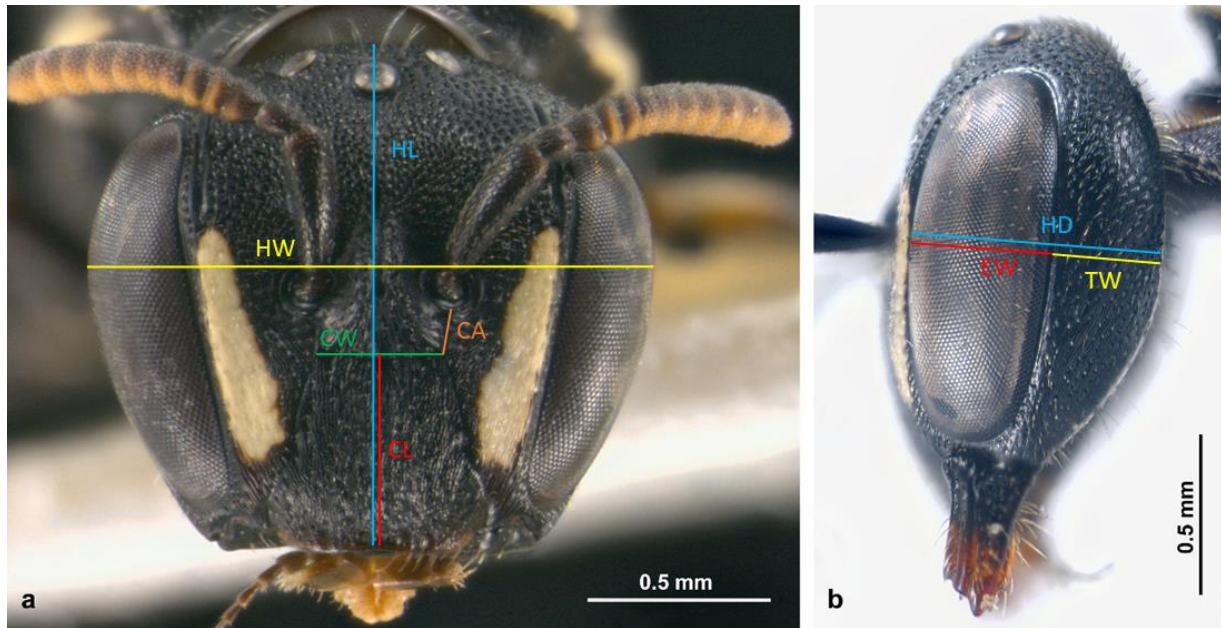


Fig. 6. Head of *H. intermedius* with illustrated measured lengths. a. Frontal view: Head length (HL, blue), head width (HW, yellow), clypeus length (CL, red), clypeus width (CW, green), and clypeus-antenna distance (CA, orange). b. Lateral view: Eye width (EW, red), temple width (TW, yellow), and head depth (HD, blue).

3. Results

3.1. DNA barcoding

According to DNA barcoding, 15 of the investigated *Hylaeus* specimens matched best with *H. brevicornis*, 16 with *H. intermedius*, ten with *H. gredleri*, and five with *H. imparilis*. Four sequences were most similar with reference sequences of *Wolbachia*, a genus of Gram-negative bacteria which parasitizes insects and other arthropods (e.g. LAUREN et al. 2008). As sequences with only one DNA strand and bad quality were excluded, only 41 sequences were used for further study.

In a Neighbor Joining and a Maximum Likelihood tree – generated with DNA barcodes from currently investigated *Hylaeus* specimens as well as *COI* sequences from BOLD – six species of the subgenus *Dentigera* are illustrated (Figs. 7, 8): *H. gredleri*, *H. intermedius*, *H. kahri*, *H. imparilis*, *H. brevicornis* and *H. glacialis*.

As expected, *H. brevicornis* and *H. imparilis* represent two well delimited clades, but one unstudied specimen of *H. glacialis* (sequence from BOLD) clusters within the clade of *H. brevicornis* (Figs. 7, 8). The DNA barcodes of *H. gredleri* describe a distinct monophylum, clearly separated from *H. intermedius*, also supported by high Bootstrap values (Figs. 7, 8). The phylogenetic trees illustrate, though, that *H. intermedius* is clustering in two groups. Both clades involve individuals from the study area. Whereas the DNA barcodes of some *H. intermedius* specimens cluster next to the clade of *H. gredleri*, the barcodes of others are placed next to *H. kahri*, a morphologically well-defined species, that was not barcoded for this study. The splitting of *H. intermedius* in two clades is strongly supported by both phylogenetic trees, but even better in the Maximum Likelihood tree with a Bootstrap value of 100 (Fig. 8). Furthermore, there is a high mean evolutionary divergence of 12% over the sequence pairs between the two groups of *H. intermedius* (Tab. 1). Also, a pairwise comparison of all sequences from this species illustrates that the evolutionary divergence is much higher between the two groups than within them. Whereas within one group of *H. intermedius* evolutionary divergence is not higher than 1.5%, it is always over 10% between the two groups (Appendix 3).

One male specimen (API 006 818) – collected on May 18, 2017 at the premises of the former Nordbahnhof by the author – could not be assigned to a distinct species, neither morphologically, nor genetically (Figs. 7, 8). It features an incomplete yellow coloration of the clypeus and broad, partly yellow scapi. This specimen has to be observed in more detail yet.

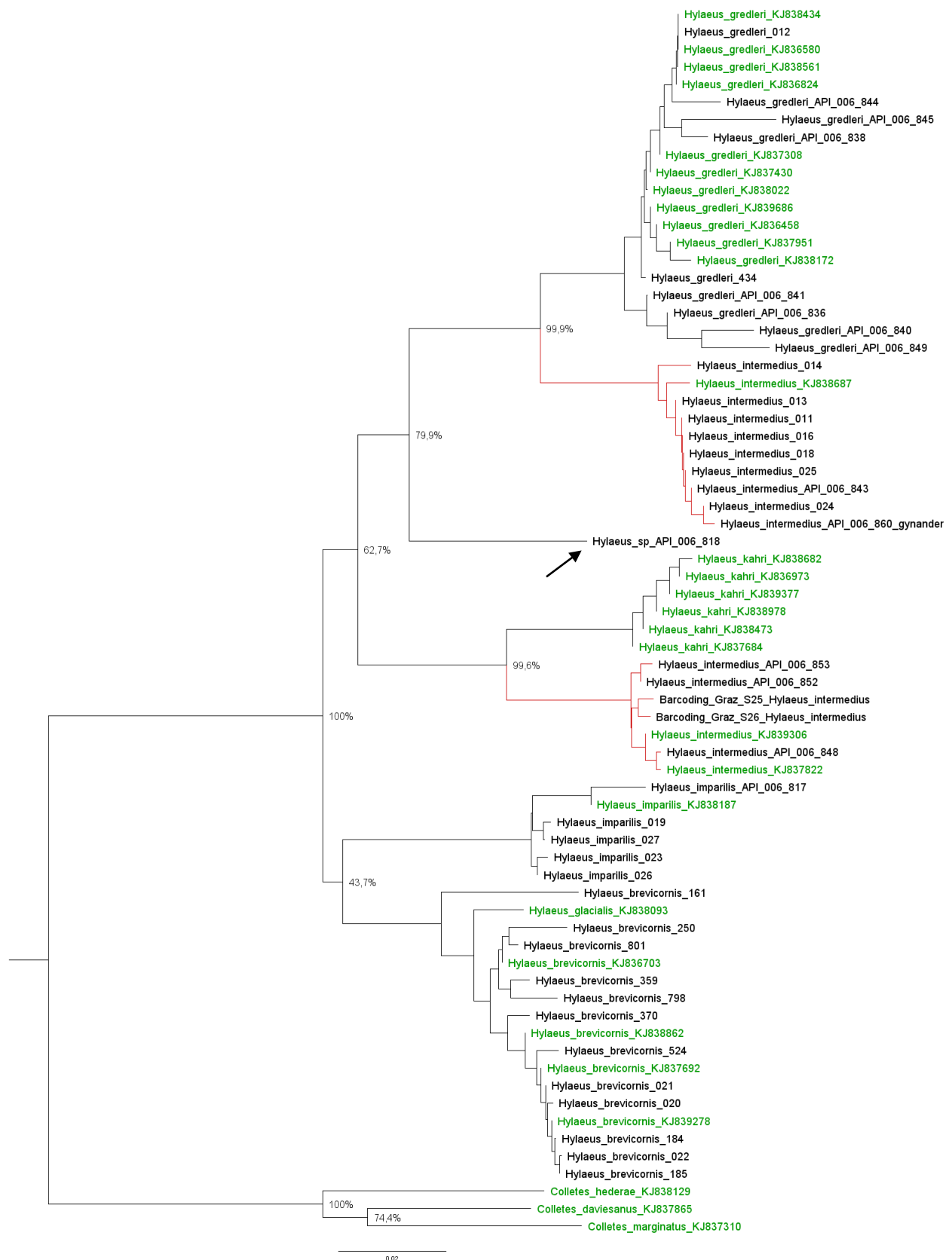


Fig. 7. Neighbor Joining tree of the investigated *Hylaeus* specimens (black) and reference data from BOLD (green), with Bootstrap values. The two separated clades of *H. intermedius* are illustrated in red. The indeterminate specimen API 006 818 is marked with an arrow.

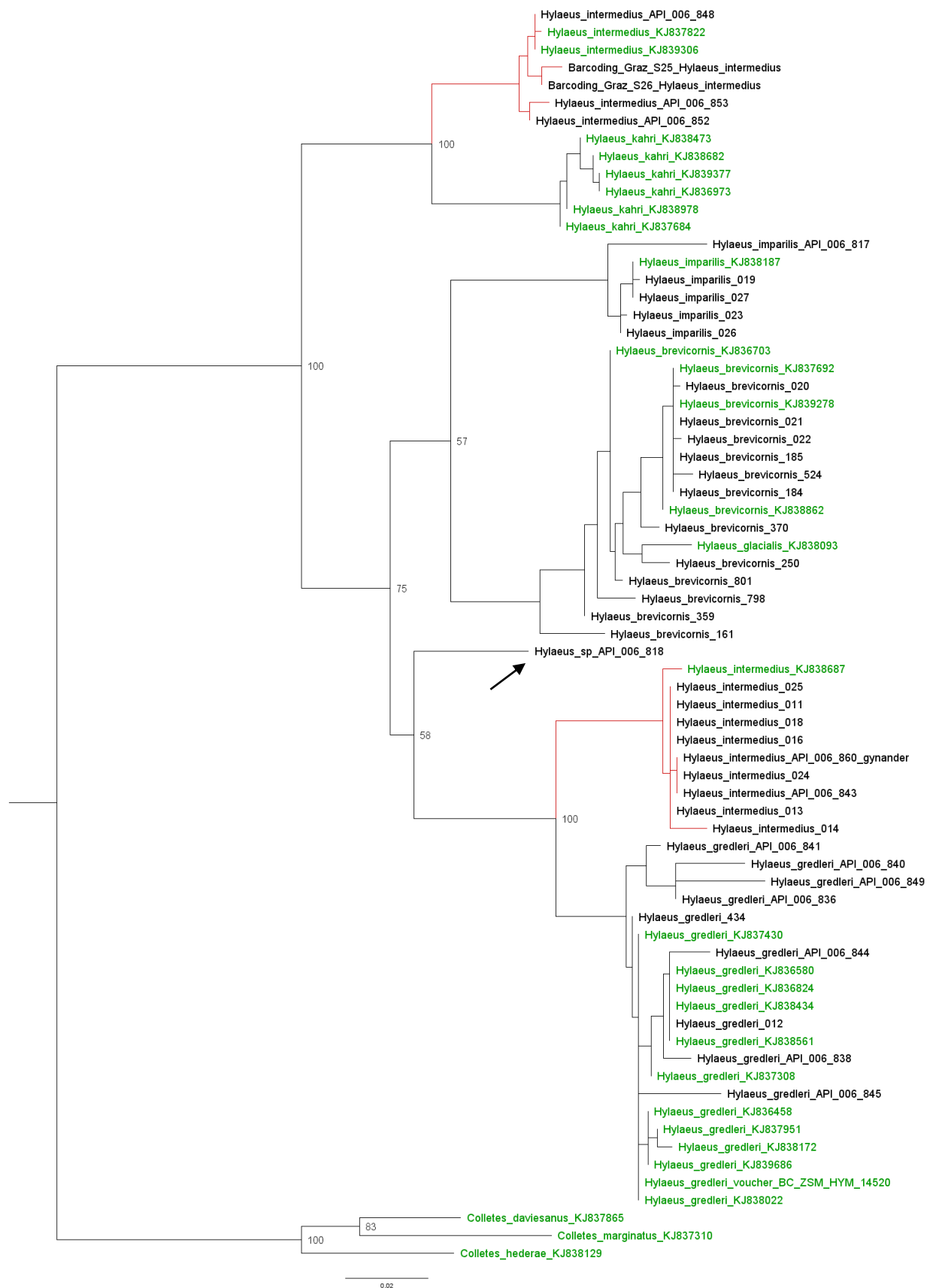


Fig. 8. Maximum Likelihood tree of the investigated specimens (black) and reference data from BOLD (green), with Bootstrap values. The two separated clades of *H. intermedius* are illustrated in red. The indeterminable specimen API 006 818 is marked with an arrow.

Table 1. Mean evolutionary divergence over sequence pairs between the two clades of *H. intermedius* (calculated with Mega7.0.25).

Species 1	Species 2	Mean Distance	Prozent
Gp 1 (<i>H. intermedius</i> Clade 1)	Gp 2 (<i>H. intermedius</i> Clade 2)	0,120	12,0%

3.2. Pollen analyses

All investigated *Hylaeus* species – *H. brevicornis*, *H. gredleri*, *H. intermedius*, and *H. imparilis* – are polylectic, collecting pollen from a variety of different plant families in the study area. However, they do prefer plants from the families Apiaceae and Brassicaceae. Almost half of the crop content was Apiaceae pollen and approximately a quarter Brassicaceae pollen (Fig. 9). At the premises of the former Nordbahnhof, *Hylaeus* was mostly caught on the Apiaceae *Daucus carota* and *Falcaria vulgaris* as well as on the Brassicaceae *Bunias orientalis*, *Barbarea vulgaris*, and *Berteroa incana* (list of flowers from Nordbahnhof: Appendix 2). Pollen of the type Asteraceae tubuliflorae were also quite highly represented with approximately 15% (Fig. 9). *Solidago canadensis* is an Asteraceae with tubuliflore pollen type at the sampling area on which *Hylaeus* was frequently caught. Especially late in the season this plant seemed to be an important pollen source for various *Hylaeus* species. While from June to August less than 5% of the crop content was represented by the pollen type Asteraceae tubuliflorae, it was almost a quarter from August to September, when *Solidago canadensis* was flowering (Fig. 10). Also, Brassicaceae pollen was collected by the investigated *Hylaeus* species more frequently late in the season (Fig. 10). The pollen type triangular/tricolporate – involving plants from Crassulaceae, Rosaceae and *Linaria* – accounted for 17% of the crop content (Fig. 9). *Hylaeus* was frequently caught on *Sedum*, *Linaria vulgaris*, *Potentilla*, and *Rubus*. Although *Rosa* was prevalent at the sampling area too, *Hylaeus* was hardly ever caught on this plant (list of flowers from Nordbahnhof: Appendix 2).

All other pollen types were represented with less than 1% of the total amount (Fig. 9). *Hypochaeris* sp. and *Hieracium* sp. – existing on the sampling area – feature pollen from the type Asteraceae liguliflorae, which accounted for only 0.1% of the crop content, and therefore are no important pollen sources for *Hylaeus*. It is similar with Caryophyllaceae, represented by *Silene vulgaris*, *Silene latifolia* and *Saponaria officinalis* on the sampling site (Fig. 9).

The investigated *Hylaeus* species are not only polylectic, but also do not show flower steadiness, as the crop content of each single individual contained pollen of different plants (Appendix 4).

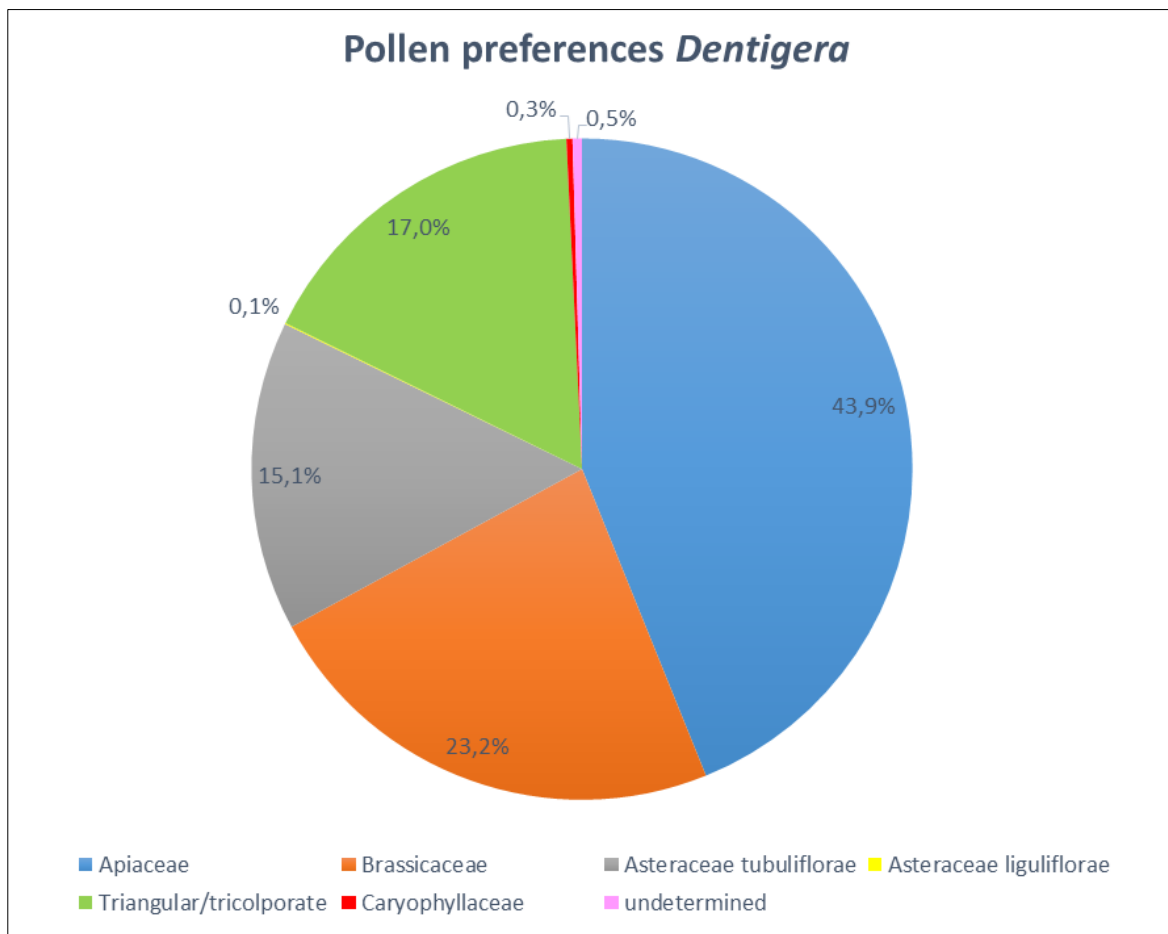


Fig. 9. Percentages of pollen in crop content of the investigated *Hylaeus* specimens on the premises of the former Nordbahnhof in Vienna.

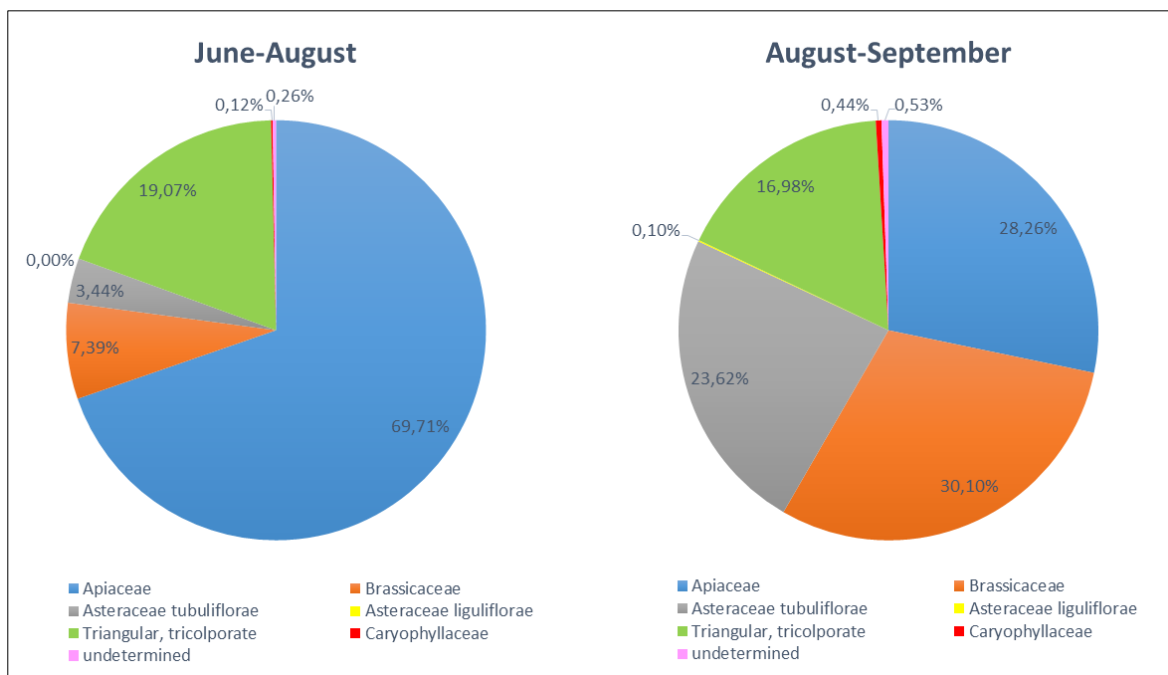


Fig. 10. Percentages of pollen in crop content of the investigated *Hylaeus* specimens from June to August and from August to September.

Within the species the pollen composition varies a lot (Appendix 4); however, between the species no essential differences were detected in their feeding preferences. All investigated *Hylaeus* species feature approximately the same pollen composition, with high percentages of Apiaceae pollen, Brassicaceae pollen, pollen of the type Asteraceae tubuliflorae, and the type triangular/tricolporate (Appendix 4). As *H. gredleri* and *H. imparilis* were only sampled rarely at the study area, a comparison of the pollen composition in the crop content was only reasonable between the species *H. brevicornis* and *H. intermedius*, illustrated in Fig. 11.

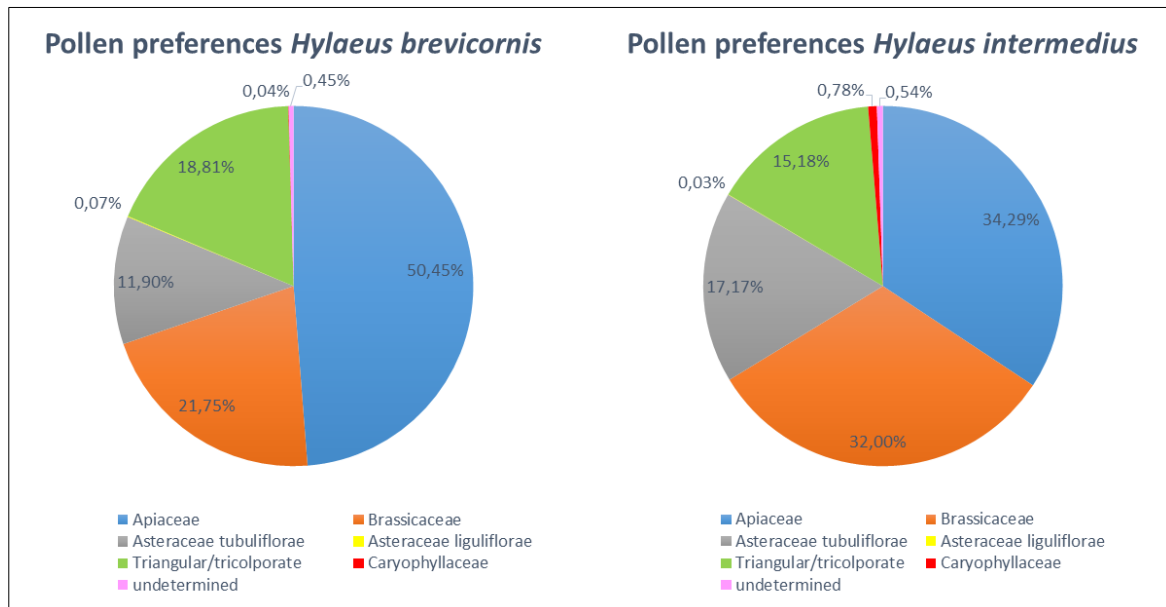


Fig. 11. Percentage of pollen in crop content of *Hylaeus brevicornis* (n = 15) and *H. intermedius* (n = 13).

3.3. Morphometric measurements

Due to intraspecific size variation, the absolute lengths in the measured females vary strongly, leading to a rather wide range from minimum to maximum values (Tab. 2). In general, *H. gredleri* and *H. intermedius* show higher absolute values (i.e., are larger) than *H. brevicornis* and *H. imparilis*. An exception is CA, which is remarkably short in most of the *H. gredleri* specimens (Tab. 2).

The mean value for the cephalic index is highest in *H. gredleri* with about 113. The cephalic index for the other three species is similar, although slightly lower in *H. imparilis* with about 108 (Tab. 2, Fig. 12). The scatter diagram illustrates that the measured specimens of *H. kahri* and two specimens of *H. intermedius* group 2 even lie above *H. gredleri*, representing a very high cephalic index (Fig. 12).

Hylaeus gredleri also features the highest mean for the clypeus index with almost 72 – representing a broader, shorter clypeus – whereas *H. imparilis* has the lowest clypeus index with only 60.7 (Tab. 2, Fig. 13).

The head depth index in *H. imparilis* and *H. brevicornis* is lower than in *H. gredleri* and *H. intermedius*. *Hylaeus gredleri* has the highest mean value for the head depth index with almost 57, whereas *H. imparilis* has a mean of only 51 (Tab. 2, Fig. 14). Again, the specimens of *H. kahri* and two specimens of *H. intermedius* group 2 lie above all other species in the scatter diagram, representing a very high head depth index (Fig. 14). Also, the mean values for the temple width index are lower in *H. imparilis* and *H. brevicornis* than in *H. gredleri* and *H. intermedius*. The differences between the species are even more pronounced for this index, with only about 99 in case of *H. imparilis* and *H. brevicornis* and approximately 120 in case of *H. gredleri* and *H. intermedius* (Tab. 2, Fig. 15).

An interesting finding was that CA varies strongly between the species, on average being smallest in *H. gredleri*, shown both by absolute values and indices (Tab. 2). Therefore, the mean of the CA index is much lower in *H. gredleri* (with only 35.7) than in *H. brevicornis* and *H. imparilis* (with over 44). *H. intermedius* lies in between with a CA index of approximately 41 (Tab. 2). However, the scatter diagram (Fig. 16) also shows that there are outliers, especially among the *H. gredleri* specimens, including the female syntype of *H. gredleri*.

The most important differences between the investigated species are summed up in Table 3.

Table 2. Morphometry of *Hylaeus*, heads of females: Mean values (M), as well as minimum and maximum values (min/max) for all indices and measurements (mm) per species.

	<i>H. brevicornis</i>	<i>H. gredleri</i>	<i>H. imparilis</i>	<i>H. intermedius</i>
M (cephalic index)	109.8	113.3	108.3	110.6
min (cephalic index)	106.0	110.3	103.3	105.7
max (cephalic index)	113.8	117.6	113.0	114.1
M (clypeus index)	64.6	71.6	60.7	64.7
min (clypeus index)	55.8	61.7	53.4	59.0
max (clypeus index)	71.7	83.3	67.3	70.0
M (head depth index)	51.9	56.8	51	53.4
min (head depth index)	49.3	52.7	47.7	50.6
max (head depth index)	54.9	63.0	43.4	55.9
M (temple width index)	99.4	119.8	99.3	122.4
min (temple width index)	85.7	109.8	86.4	112.2
max (temple width index)	118.9	126.3	121.2	136.5
M (CA index)	44.5	35.7	47.7	40.9
min (CA index)	41.7	30.2	44.0	37.3
max (CA index)	47.8	45.7	52.4	45.8
M (HL)	1.285	1.325	1.284	1.429
min (HL)	1.219	1.202	1.185	1.330
max (HL)	1.365	1.485	1.382	1.511
M (HW)	1.411	1.503	1.390	1.581
min (HW)	1.384	1.382	1.305	1.408
max (HW)	1.554	1.674	1.511	1.717
M (CL)	0.453	0.475	0.479	0.539
min (CL)	0.395	0.412	0.412	0.481
max (CL)	0.498	0.567	0.524	0.601
M (CW)	0.292	0.340	0.290	0.349
min (CW)	0.249	0.309	0.258	0.309
max (CW)	0.326	0.369	0.326	0.369

M (EW)	0.334	0.341	0.331	0.343
min (EW)	0.309	0.326	0.283	0.318
max (EW)	0.361	0.361	0.378	0.378
M (TW)	0.332	0.405	0.327	0.422
min (TW)	0.309	0.378	0.309	0.378
max (TW)	0.378	0.455	0.361	0.472
M (HD)	0.666	0.746	0.657	0.765
min (HD)	0.627	0.704	0.618	0.704
max (HD)	0.704	0.815	0.704	0.824
M (CA)	0.129	0.118	0.135	0.136
min (CA)	0.115	0.103	0.122	0.122
max (CA)	0.147	0.135	0.141	0.147

Table 3. Morphometric measurements relevant for species characterization of females of *H. brevicornis*, *H. gredleri*, *H. imparilis*, and *H. intermedius*.

	Cephalic index	Clypeus index	Head depth index/ temple width index	CA index
<i>Hylaeus brevicornis</i>	low	intermediate	low	high
<i>Hylaeus gredleri</i>	high	very high	high	very low (but outliers)
<i>Hylaeus imparilis</i>	very low	very low	low	very high
<i>Hylaeus intermedius</i>	rather high	intermediate	high	intermediate

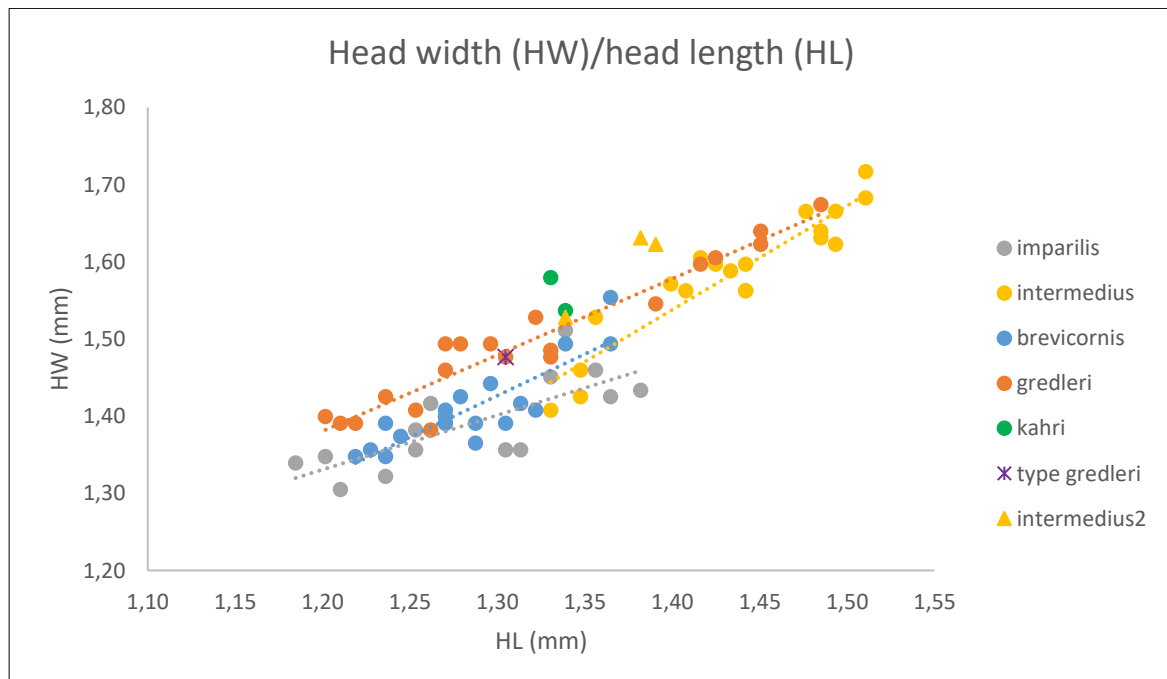


Fig. 12. Head width (HL) to head length (HL) in mm, with trend lines, for the four investigated *Hylaeus* species (*H. brevicornis*, *H. gredleri*, *H. imparilis*, and *H. intermedius*) plus the type specimens of *H. gredleri*, two specimens of *H. kahri*, and the three females of *H. intermedius* group 2.

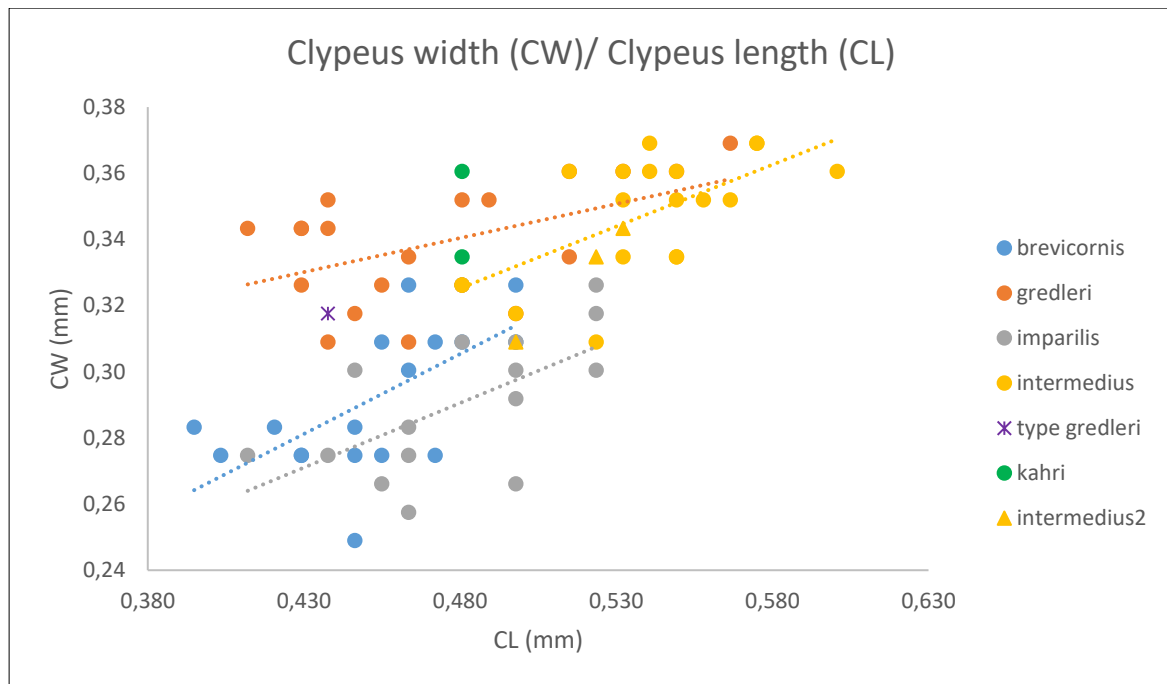


Fig. 13. Clypeus width (CW) to clypeus length (CL) in mm, with trend lines, for the four investigated *Hylaeus* species plus the type specimen of *H. gredleri*, two specimens of *H. kahri*, and the three females of *H. intermedius* group 2.

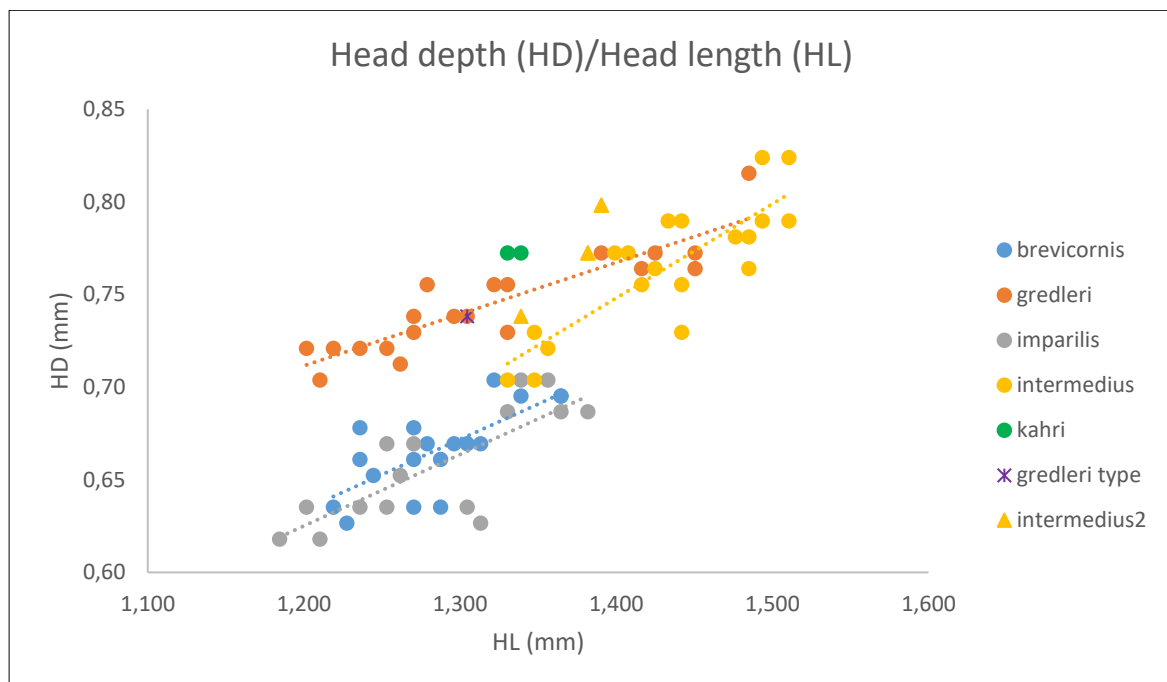


Fig. 14. Head depth (HD) to head length (HL) in mm, with trend lines, for the four investigated *Hylaeus* species plus the type specimen of *H. gredleri*, two specimens of *H. kahri*, and the three females of *H. intermedius* group 2.

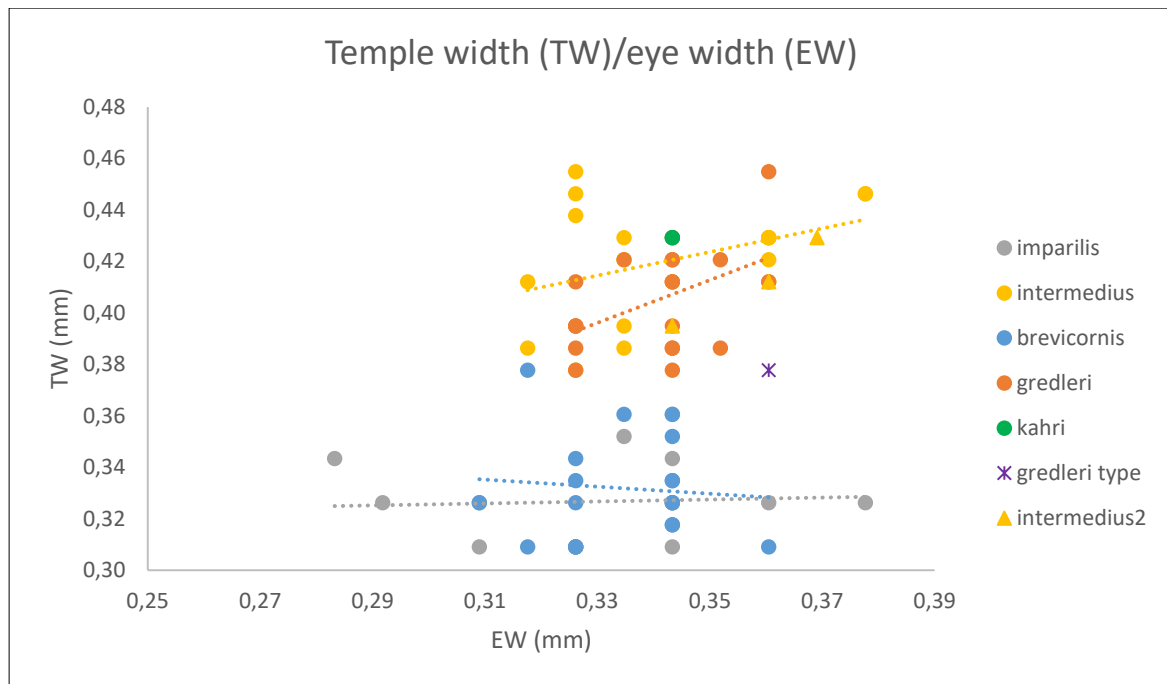


Fig. 15. Temple width (TW) to eye width (EW) in mm, with trend lines, for the four investigated *Hyaenus* species plus the *H. gredleri* type specimen, two *H. kahri* specimens, and the three females of *H. intermedius* group 2.

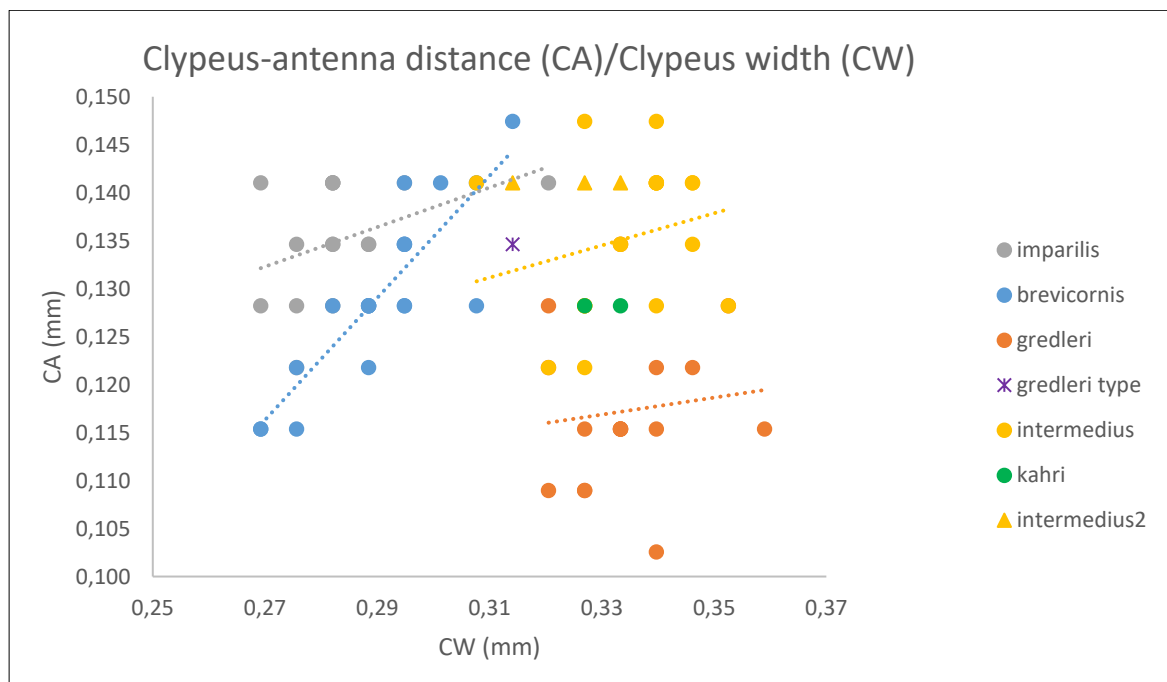


Fig. 16. Clypeus-antenna distance (CA) to clypeus width (CW) in mm, with trend lines, for the four investigated *Hyaenus* species plus the *H. gredleri* type specimen, two *H. kahri* specimens, and the three females of *H. intermedius* group 2.

All measured parameters of the head – HL, HW, CL, CW, CA, EW, TW, and HD – were log-transformed and pooled in a principal component analysis. The first principal component (PC1) explains over 65% of the total variance, PC2 explains approximately 20% (Tab. 4). Based on the loadings of the individual parameters (Tab. 5), PC1 can be considered a general size-axis, while PC2 is most strongly based on differences in CA.

Whereas the plots for *H. brevicornis* and *H. imparilis* cluster considerably, the plots for *H. gredleri* are more separated, but overlapping does occur with the species *H. intermedius* (Fig. 17). The measured syntype of *H. gredleri* lies slightly distant from the other *H. gredleri* specimens. The three females of *H. intermedius* group 2 and the *H. kahri* specimens also cluster within *H. intermedius* (Fig. 17).

Table 4. Eigenvalues, percentage of explained variance and confidence intervals of the first three principal components based on 1000 Bootstrap resamplings.

PC	Eigenvalue	% variance	Eig 2.5%	Eig 97.5%
1	0.00783	65.29	58.02	72.45
2	0.00243	20.27	13.51	26.83
3	0.00068	5.68	4.17	7.42

Table 5. Loadings of the first 3 principal components for eight morphometric measurements.

	PC1	PC2	PC3
HL (log)	0.268	0.225	-0.118
HW (log)	0.324	0.111	-0.054
CL (log)	0.368	0.309	-0.742
CW (log)	0.449	-0.174	0.118
CA (log)	-0.009	0.877	0.433
EW (log)	0.137	0.056	-0.174
TW (log)	0.578	-0.184	0.427
HD (log)	0.366	-0.066	0.138

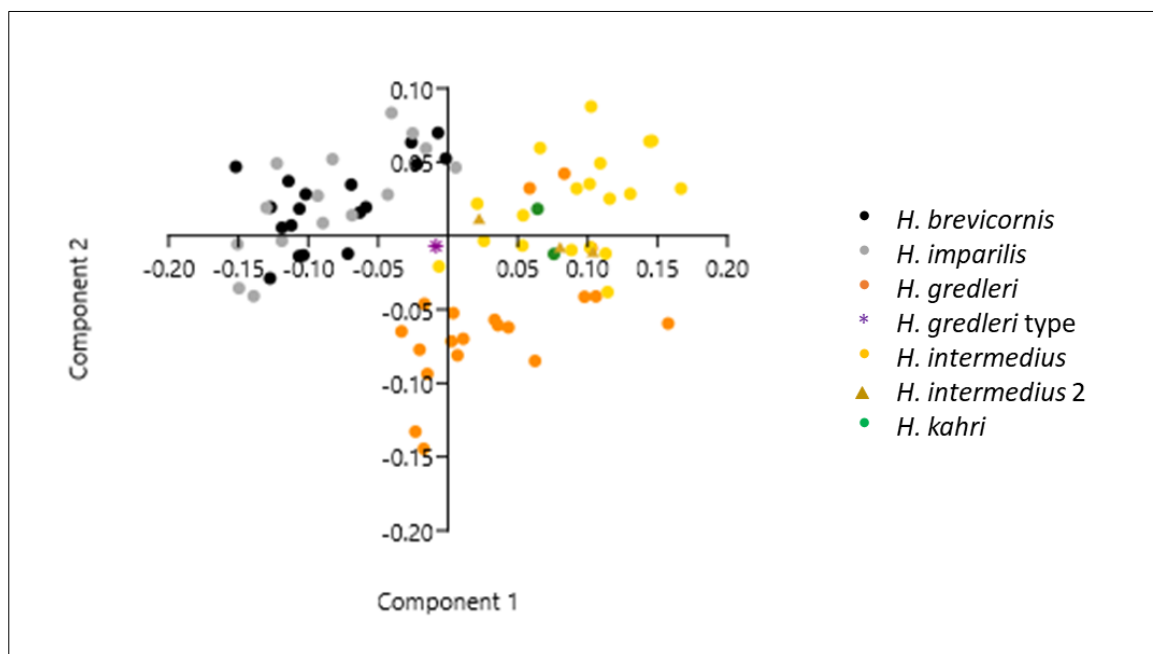


Fig. 17. Principal components 1 and 2 based on eight morphometric measurements (HL, HW, CL, CW, EW, TW, HD, and CA) of all examined *Hylaeus* specimens.

4. Discussion

Two of the three approaches applied in this study – genetic and morphometric analyses – do provide congruent results. Insights gained by pollen analyses are not really helpful for solving the taxonomic problem as no differences between the species were detected. All of them are polylectic with a preference for Apiaceae and Brassicaceae at the sampling area.

Molecular analyses confirmed delimitation on basis of morphology in the species *H. brevicornis*, *H. gredleri*, *H. imparilis*, and *H. kahri*, but raised the question of a cryptic species in *H. intermedius*, as the specimens of the sample obviously split up in two clades.

Although high intraspecific variations complicate a reliable determination based on morphology, morphometric measurements do largely support current species delimitation. Further morphometric measurements in the light of the arisen two-species-hypothesis of *H. intermedius*, including also males, may help to detect subtle morphological differences. Besides, additional molecular analyses, investigating more specimens and other gene sequences – also nuclear gene sequences – shall clarify the ambiguity in the species *H. intermedius*.

4.1. DNA barcoding

All sequences, except one (API 006 818, cf. 3.1.), could be matched successfully with reference sequences from BOLD. In order to exclude potentially wrongly determined species in BOLD, already published data were preferentially used for comparison and further study. Many of the published reference data in BOLD for the genus *Hylaeus* were uploaded by SCHMIDT et al. (2015). However, for some specimens no public reference sequences were available yet. In such cases the DNA barcode is entered in the database, but neither the person who uploaded the sequence, nor the collecting data of the specimen is detectable. If the majority of hits in BOLD belonged to one species with high percentages and only one, not public barcode of a different species was shown, wrong determination can be assumed. For example, this was the case with *H. intermedius* several times: Whereas the generated *COI* sequence matched best with several published reference sequences of “*H. intermedius*”, there were also matches with sequences of “*H. kahri*” or “*H. gredleri*”; however, these DNA barcodes were not public. As *H. intermedius* was only recently reintroduced as a distinct species, wrong determination is easily possible. In other databases like NCBI (National Center for Biotechnology Information)³ the species *H. intermedius* is not represented yet and therefore

³ <https://www.ncbi.nlm.nih.gov/>, November 26, 2017

only *COI* sequences of “*H. gredleri*” were shown when trying to match a sequence of *H. intermedius* with DNA barcodes in NCBI.

Beside a Neighbor-Joining tree, a Maximum-Likelihood tree was generated, to compare the outcome in both cases. Whereas there were some minor differences, the essential conclusions stayed the same. Most importantly, *H. intermedius* – the recently reintroduced *Hylaeus* species (SCHMIDT et al. 2015, DATHE et al. 2016) – obviously clustered in two different clades in both phylogenetic trees. Therefore *H. intermedius* in the present sense is supposed to include a cryptic species. Bootstrap values highly support this outcome, especially in case of the Maximum Likelihood tree with a Bootstrap of 100. Already in the study of SCHMIDT et al. (2015) the investigated specimens identified as *H. intermedius* split up in two groups; however, they originated from different parts in Europe. Whereas the specimens which cluster next to *H. gredleri* originated from Turkey (S Aegaeis) and France (Provence-Alpes-Côte d’Azur), the specimens which cluster next to *H. kahri* originated from Italy (Aosta Valley and Veneto) (SCHMIDT et al. 2015). In the present study specimens belonging to both clades were caught in Vienna, most of them from one and the same sampling site, the premises of the former Nordbahnhof. Therefore, differences due to geographic distances can be excluded. During first morphological observations only small differences between the two groups of *H. intermedius* could be detected in females (cf. 3.3. and 4.3. Morphometric measurements). However, males of *H. intermedius* clustering next to *H. kahri* were bigger sized and had surprisingly broad scapi, thus did neither fit the key characters in DATHE et al. (2016) nor agreed with the holotype (*H. Zettel*, pers. comm.). On the other hand they clearly differ from *H. kahri* by small tubercles on gaster sternite 3 (compare DATHE et al. 2016).

Hylaeus gredleri clearly separated from *H. intermedius* in the generated phylogenetic trees, corroborating the work of DATHE et al. (2016), in which *H. intermedius* was dissociated from *H. gredleri* by morphological features.

The monophyly of both *H. brevicornis* and *H. imparilis* can be confirmed. This was expected, as the two species are relatively easy to determine, also by morphology. The *COI* sequences of several *H. imparilis* individuals also matched with a reference sequence from “*H. styriacus*” in BOLD whose DNA barcode was not public yet; again, this sequence was not considered, as no data were available to check correct determination. Interestingly, the single reference sequence of “*H. glacialis*” in BOLD clustered within *H. brevicornis* in both generated phylogenetic trees. Likely this specimen was wrongly determined. As there was only one reference sequence in BOLD, further investigations are necessary.

Some of the *Hylaeus* specimens used for pollen analyses and for morphometric analyses were not successfully determined genetically. In such cases, determination was done on basis of

morphology only. However, the genetically analyzed specimens served as references, facilitating a more accurate morphological determination, especially of females.

4.2. Pollen analyses

No analyses of the crop content were carried out for the genus *Hylaeus* in Europe yet, except for SCHODER & WIESBAUER (2017). Similar studies are only available from the United States (e.g. WILSON et al. 2010). Previous data about feeding preferences of *Hylaeus* (WESTRICH 1989, SCHEUCHL & WILLNER 2016) are mainly based on observations, as analyses of the crop content require time consuming dissections. Therefore, results gained by pollen analyses in my thesis are unique for the genus *Hylaeus* in Europe. Diversity of the pollen content allows to conclude a possible ecological niche partitioning by flower choices.

The investigated *Hylaeus* specimens turned out to be polylectic, thus confirming previous literature (WESTRICH 1989, SCHEUCHL & WILLNER 2016). The crop content of most individuals consisted of a mixture of pollen from different plant groups, using several pollen sources at one and the same collecting flight – this means that they do not show flower steadiness.

Strong preferences for plants with small or compound florescences like Apiaceae, Brassicaceae, *Sedum* and *Solidago* were detected for the investigated species. Generally, *Hylaeus* was rarely caught on Lamiaceae, Fabaceae or Boraginaceae. *Echium vulgare* was very common at the sampling site and many bee species could be observed there, but almost no masked bees. However, in literature *Echium vulgare* is mentioned to be a good pollen source for *Hylaeus* (WESTRICH 1989). Further observations on *Echium* shall clarify this inconsistency in the next years. Masked bees were also observed while collecting on *Linaria vulgaris*; to get pollen out of the closed-lipped florescence they completely crawled into it. Pollen from this plant was found in the crop content of several specimens with high reliability.

It can be summed up that the investigated *Hylaeus* species are generalists, seemingly not showing differences in their larval feeding preferences. It is supposed that the four closely related species *H. gredleri*, *H. intermedius*, *H. brevicornis*, and *H. imparilis* differ slightly in their preferred microclimates. *Hylaeus gredleri* prefers more humid habitats like meadows, light forest or forest glades, but also waste land and settlements (SCHEUCHL & WILLNER 2016). *Hylaeus imparilis* is much more common in Mediterranean parts of Europe than in Central Europe (SCHEUCHL & WILLNER 2016), and rather rarely collected in Austria. Also, *H. intermedius* seem to prefer warmer and drier places (pers. obs.). *Hylaeus brevicornis* does not have a distinct habitat association, seemingly tolerating a wide range of different microclimates (SCHEUCHL & WILLNER 2016). It makes sense that at the premises of the former Nordbahnhof in Vienna – a very dry and warm place (SCHINNINGER et al. 2002) – *H. intermedius* was more

frequently caught than *H. gredleri*. At other sites near Vienna, though, it was the other way round.

4.3. Morphometric measurements

The *Hylaeus brevicornis* group is still challenging to distinguish by means of morphology. This concerns especially females. In existing determination keys (DATHE 1980, AMIET et al. 1999, DATHE et al. 2016) head proportions are used to describe differences between the species. As no exact measurements are available in literature, the author aimed at generating measuring data for the species *H. brevicornis*, *H. gredleri*, *H. imparilis*, and *H. intermedius*, in order to detect potential differences.

Beside the four investigated *Hylaeus* species, two females of *H. kahri* were measured to obtain a more complete overview of the *Hylaeus brevicornis* group. Furthermore, three *H. intermedius* females which split up in the phylogenetic tree and cluster next to *H. kahri* – called *H. intermedius* group 2 in this study – are illustrated separately in the scatter diagrams. For these specimens, no mean values and indices were calculated, as no meaningful data can be generated for only two or three measured individuals, but differences were also detected by means of the scatter diagrams.

As especially discrimination of female individuals is difficult, and this sex was also relevant for pollen analyses, only females were measured during this study. Intraspecific and interspecific size differences were found within the measured individuals, therefore indices are more informative than absolute values. The low absolute values within *H. brevicornis* and *H. imparilis* evidence that these species are on average smaller sized.

As expected due to descriptions in literature (e.g. AMIET et al. 1999, DATHE et al. 2016), *H. gredleri* features a high cephalic index and *H. imparilis* the lowest, but measurable differences were rather small. It can be concluded that it is not only the width itself which matters, but the head shape, which cannot be detected with classical morphometry and would require a geometrical morphometry approach. The females of *H. kahri* and two specimens of *H. intermedius* group 2 obviously feature the broadest heads, clearly seen in the scatter diagram (Fig. 12). This could be a character to distinguish the two clades of *H. intermedius*, making sense in the light of the proximity between the clades *H. kahri* and *H. intermedius* group 2. Also head depth (relative to head length) is very high in *H. kahri* and *H. intermedius* group 2 compared to the other species. But this is again only the case for two of the three measured females of *H. intermedius* group 2. Generally, *H. gredleri*, *H. kahri*, and both groups of *H. intermedius* have a much higher temple width than *H. brevicornis* and *H. imparilis*, making their heads appear bulkier. Another feature for species delimitation is the clypeus width. Although

overlapping does occur, the clypeus index is obviously highest in *H. gredleri* and *H. kahri*. This goes along with the assumption that *H. gredleri* has the maximal head width lower in the face, and their heads appear rounder. At first sight also the clypeus-antenna distance seems to be a good character for species delimitation, with *H. gredleri* showing the lowest CA values. The female syntype of *H. gredleri* deviates from the other *H. gredleri* specimens in this measured length, though, raising doubts about the conspecificity of the type series. Another possible explanation is variance between populations due to geographical distances, since the syntype of *H. gredleri* was not collected in Austria.

Although differences between the investigated species were detected, rather large overlaps occurred, illustrated by highly divergent minimal and maximal values. The species *H. brevicornis* especially clusters with *H. imparilis* in the scatter diagrams as well as the principal component analysis (Fig. 12–17). These two species are easier to distinguish due to coloration and puncturation, as described in literature (DATHE 1980, AMIET et al. 1999, DATHE et al. 2016). It was also expected that *H. imparilis* features slimmer and longer heads and a slimmer clypeus, but among the measured females, differences to *H. brevicornis* were only subtle concerning these parameters.

Problematic are the overlaps between the females of *H. gredleri*, *H. kahri*, and both clades of *H. intermedius* in the scatter diagrams and the principal component analysis (Fig. 12–17). Coloration and puncturation of their faces do not vary remarkably. Further morphometric studies shall be carried out in order to find additional distinguishing features for those species – including also male individuals, which possess more distinct characters, and both clades of *H. intermedius*.

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6. Appendix

Appendix 1. All investigated *Hylaeus* specimens, including species, sex, identification number, as well as collector, collecting date and applied approaches for each individual. Numbers given specifically for this study are listed in red, numbers given in the course of the project “Genetische Vielfalt der Wildbienen Österreichs” in green and numbers given by H. Zettel in black.

Species	Sex	Number	Location	Collector	Collecting date	Approaches
<i>Hylaeus brevicornis</i>	female	API 006 456 (7)	Nbhf 1	S. Schoder	24.08.2016	Pollen analyses, DNA barcode
<i>Hylaeus brevicornis</i>	female	API 006 457 (8)	Nbhf 2	S. Schoder	24.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 006 458 (9)	Nbhf 2	S. Schoder	24.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 006 459 (10)	Nbhf 1	S. Schoder	25.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 006 460 (15)	Nbhf 2	S. Schoder	25.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 006 462 (20)	Nbhf 2	S. Schoder	25.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 006 463 (21)	Nbhf 1	S. Schoder	29.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 006 461 (22)	Nbhf 2	S. Schoder	01.09.2016	DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	31	Nbhf 3	S. Schoder	01.06.2017	Pollen analyses
<i>Hylaeus brevicornis</i>	female	API 005 035 (92)	Wien, Stammersdorf	D. Zimmermann	08.06.2016	DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 005 043 (161)	Nbhf 4	S. Schoder	23.06.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 005 040 (184)	Nbhf 4	D. Zimmermann	23.06.2016	DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 005 041 (370)	Nbhf 2	D. Zimmermann	09.08.2016	Pollen analyses, Morphometry
<i>Hylaeus brevicornis</i>	female	API 005 042 (185)	Nbhf 4	D. Zimmermann	23.06.2016	DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 005 036 (200)	Nbhf 4	S. Schoder	30.06.2016	Morphometry
<i>Hylaeus brevicornis</i>	female	API 005 032 (447)	Nbhf 4	S. Schoder	04.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 005 037 (250)	Nbhf 1	D. Zimmermann	11.07.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 005 038 (359)	Nbhf 1	S. Schoder	09.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	API 005 034 (706)	Nbhf 1	S. Schoder	14.09.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	798	NÖ, Eichkogel	D. Zimmermann	14.09.2016	DNA barcode
<i>Hylaeus brevicornis</i>	female	API 005 033 (524)	Nbhf 4	S. Schoder	25.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus brevicornis</i>	female	801	NÖ, Eichkogel	D. Zimmermann	14.09.2016	DNA barcode
<i>Hylaeus gredleri</i>	female	171	Nbhf 2	D. Zimmermann	23.06.2016	Pollen analyses
<i>Hylaeus gredleri</i>	female	API 006 476 (12)	Nbhf 2	S. Schoder	24.08.2016	Pollen analyses, DNA barcode, Morphometry

<i>Hylaeus gredleri</i>	female	API 006 832	Nbhf 5	H. Zettel	02.07.2016	Morphometry
<i>Hylaeus gredleri</i>	female	API 006 833	Nbhf 5	H. Zettel	02.07.2016	Morphometry
<i>Hylaeus gredleri</i>	female	API 006 834	Nbhf 5	H. Zettel	02.07.2016	Morphometry
<i>Hylaeus gredleri</i>	female	API 006 835	Nbhf 5	H. Zettel	02.07.2016	Morphometry
<i>Hylaeus gredleri</i>	female	API 006 836	Nbhf 5	H. Zettel	02.07.2016	DNA barcode, Morphometry
<i>Hylaeus gredleri</i>	female	API 006 837	Nbhf 6	H. Zettel	02.07.2016	Morphometry
<i>Hylaeus gredleri</i>	female	API 006 838	Nbhf 6	H. Zettel	02.07.2016	DNA barcode, Morphometry
<i>Hylaeus gredleri</i>	female	API 006 839	Nbhf 6	H. Zettel	02.07.2016	Morphometry
<i>Hylaeus gredleri</i>	female	API 006 840	Nbhf 4	H. Zettel	04.07.2016	DNA barcode, Morphometry
<i>Hylaeus gredleri</i>	female	API 006 841	Nbhf 1	H. Zettel	16.08.2016	DNA barcode, Morphometry
<i>Hylaeus gredleri</i>	female	API 006 842	Nbhf 2	H. Zettel	03.08.2016	Morphometry
<i>Hylaeus gredleri</i>	female	API 006 844	Wien, Donaupark	H. Zettel	30.07.2016	DNA barcode, Morphometry
<i>Hylaeus gredleri</i>	female	API 006 845	Wien, Stammersdorf	H. Zettel	03.09.2016	DNA barcode, Morphometry
<i>Hylaeus gredleri</i>	female	369	Nbhf 2	D. Zimmermann	09.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus gredleri</i>	female	484	Nbhf 4	S. Schoder	18.08.2016	Pollen analyses, Morphometry
<i>Hylaeus gredleri</i>	female	434	Nbhf 1	S. Schoder	04.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus gredleri</i>	female	API 006 849	Nbhf 5	H. Zettel	04.07.2016	DNA barcode, Morphometry
<i>Hylaeus gredleri</i>	female	211	Nbhf 2	S. Schoder	30.06.2016	Pollen analyses, Morphometry
<i>Hylaeus gredleri</i>	female	171	Nbhf 2	D. Zimmermann	23.06.2016	Pollen analyses, Morphometry
<i>Hylaeus imparilis</i>	female	API 006 472 (19)	Nbhf 1	S. Schoder	24.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus imparilis</i>	female	API 006 473 (23)	Nbhf 4	S. Schoder	24.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus imparilis</i>	female	API 006 474 (26)	Nbhf 2	S. Schoder	25.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus imparilis</i>	female	API 006 475 (27)	Nbhf 1	S. Schoder	29.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus imparilis</i>	female	API 004 014	Nbhf 1	H. Zettel	03.08.2016	Morphometry
<i>Hylaeus imparilis</i>	female	API 004 015	Nbhf 2	H. Zettel	03.08.2016	Morphometry
<i>Hylaeus imparilis</i>	female	API 004 016	Nbhf 7	H. Zettel	03.08.2016	Morphometry
<i>Hylaeus imparilis</i>	female	API 001 605	Wien 1110, Haidestr.	H. Zettel	28.08.2015	Morphometry
<i>Hylaeus imparilis</i>	female	API 001 604	Wien 1110, Haidestr.	H. Zettel	28.08.2015	Morphometry
<i>Hylaeus imparilis</i>	female	-	NÖ, Spitzerberg	H. Zettel	14.08.2003	Morphometry
<i>Hylaeus imparilis</i>	female	-	NÖ, Hundsheimer Berg	H. Zettel	12.08.2008	Morphometry

<i>Hylaeus imparilis</i>	female	-	NÖ, Hundsheimer Berg	H. Zettel	24.08.2006	Morphometry
<i>Hylaeus imparilis</i>	female	-	NÖ, Retz, Golitsch	H. Zettel, H. Wiesbauer	27.08.2005	Morphometry
<i>Hylaeus imparilis</i>	female	-	Retz, Windmühle	H. Zettel	17.08.2012	Morphometry
<i>Hylaeus imparilis</i>	female	-	Retz, Windmühle	H. Zettel	17.08.2012	Morphometry
<i>Hylaeus imparilis</i>	male	API 006 817	Nbhf 3	S. Schoder	18.05.2017	DNA barcode
<i>Hylaeus intermedius</i>	female	API 006 465 (11)	Nbhf 1	S. Schoder	24.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus intermedius</i>	female	API 006 464 (13)	Nbhf 2	S. Schoder	24.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus intermedius</i>	female	API 006 468 (14)	Nbhf 4	S. Schoder	24.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus intermedius</i>	female	API 006 467 (16)	Nbhf 4	S. Schoder	24.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus intermedius</i>	female	API 006 471 (17)	Nbhf 2	S. Schoder	25.08.2016	Pollen analyses, Morphometry
<i>Hylaeus intermedius</i>	female	API 006 466 (18)	Nbhf 2	S. Schoder	25.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus intermedius</i>	female	API 006 469 (24)	Nbhf 4	S. Schoder	24.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus intermedius</i>	female	API 006 470 (25)	Nbhf 2	S. Schoder	25.08.2016	Pollen analyses, DNA barcode, Morphometry
<i>Hylaeus intermedius</i>	female	28	Nbhf 3	S. Schoder	02.06.2017	Pollen analyses
<i>Hylaeus intermedius</i>	female	409	Nbhf 2	S. Schoder	09.08.2016	Pollen analyses, Morphometry
<i>Hylaeus intermedius</i>	female	360	Nbhf 1	S. Schoder	09.08.2016	Pollen analyses, Morphometry
<i>Hylaeus intermedius</i>	female	433	Nbhf 1	S. Schoder	04.08.2016	Pollen analyses, Morphometry
<i>Hylaeus intermedius</i>	female	561	Nbhf 4	S. Schoder	01.09.2016	Pollen analyses, Morphometry
<i>Hylaeus intermedius</i>	female	398	Nbhf 2	S. Schoder	09.08.2016	Pollen analyses, Morphometry
<i>Hylaeus intermedius</i>	female	API 006 843	Nbhf 6	H. Zettel	02.07.2016	DNA barcode, Morphometry
<i>H. intermedius gynander</i>	male/female	API 006 860	Nbhf 2	H. Zettel	03.08.2016	DNA barcode, Morphometry
<i>Hylaeus intermedius</i>	female	API 006 830	Nbhf 2	H. Zettel	16.08.2016	DNA barcode
<i>Hylaeus intermedius</i>	female	API 006 848	Nbhf 4	H. Zettel	01.09.2016	DNA barcode
<i>Hylaeus intermedius</i>	female	API 006 827	Nbhf 3	H. Zettel	03.08.2016	Morphometry
<i>Hylaeus intermedius</i>	female	API 006 824	Nbhf 4	H. Zettel	01.09.2016	Morphometry
<i>Hylaeus intermedius</i>	female	API 006 821	Nbhf 2	H. Zettel	03.08.2016	Morphometry
<i>Hylaeus intermedius</i>	female	579	Nbhf 3	S. Schoder	08.09.2016	Morphometry
<i>Hylaeus intermedius</i>	female	API 006 852	Nbhf 4	H. Zettel	04.07.2016	DNA barcode, Morphometry
<i>Hylaeus intermedius</i>	female	API 006 853	Nbhf 2	H. Zettel	16.08.2016	DNA barcode, Morphometry

<i>Hylaeus intermedius</i>	female	API 006 848	Nbhf 4	H. Zettel	01.09.2016	DNA barcode, Morphometry
<i>Hylaeus intermedius</i>	male	-	Nbhf 3	S. Schoder	15.06.2017	DNA barcode
<i>Hylaeus intermedius</i>	male	-	Wien, Bot. Garten	S. Schoder	20.06.2017	DNA barcode
<i>Hylaeus ??</i>	male	API 006 818	Nbhf 3	S. Schoder	18.05.2017	DNA barcode
<i>Hylaeus kahri</i>	female	-	Wien, Perchtoldsdorfer Heide	H. Zettel	05.08.2012	Morphometry
<i>Hylaeus kahri</i>	female	-	Wien, Perchtoldsdorfer Heide	H. Zettel	11.07.2012	Morphometry
<i>H. gredleri</i> syntype	female	-	-	-	-	Morphometry

Appendix 2. List of potential pollen sources for bees on the premises of the former Nordbahnhof Vienna (Noted 2016 and 2017 by the author).

Family	Species	Family	Species
Asteraceae	<i>Achillea millefolium</i>	Asteraceae	<i>Hypochaeris</i> sp.
Boraginaceae	<i>Anchusa officinalis</i>	Fabaceae	<i>Lathyrus tuberosus</i>
Apiaceae	<i>Anthriscus sylvestris</i>	Brassicaceae	<i>Lepidium draba</i>
Lamiaceae	<i>Ballota nigra</i>	Plantaginaceae	<i>Linaria vulgaris</i>
Brassicaceae	<i>Barbarea vulgaris</i>	Fabaceae	<i>Medicago sativa</i>
Brassicaceae	<i>Berteroa incana</i>	Fabaceae	<i>Melilotus albus</i>
Brassicaceae	<i>Bunias orientalis</i>	Fabaceae	<i>Melilotus officinalis</i>
Brassicaceae	<i>Capsela bursa-pastoris</i>	Fabaceae	<i>Onobrychis viciifolia</i>
Asteraceae	<i>Carduus</i> sp.	Oxalidaceae	<i>Oxalis</i> sp.
Asteraceae	<i>Centaurea stoebe</i>	Rosaceae	<i>Potentilla argentea</i>
Caryophyllaceae	<i>Cerastium fontanum</i>	Rosaceae	<i>Potentilla reptans</i>
Papaveraceae	<i>Chelidonium majus</i>	Resedaceae	<i>Reseda lutea</i>
Asteraceae	<i>Cirsium arvense</i>	Fabaceae	<i>Robinia pseudoacacia</i>
Asteraceae	<i>Cirsium vulgare</i>	Rosaceae	<i>Rosa</i> sp.
Ranunculaceae	<i>Clematis vitalba</i>	Rosaceae	<i>Rubus</i> sp.
Fabaceae	<i>Coronilla varia</i>	Adoxaceae	<i>Sambuca nigra</i>
Asteraceae	<i>Crepis biennis</i>	Caryophyllaceae	<i>Saponaria officinalis</i>
Apiaceae	<i>Daucus carota</i>	Crassulaceae	<i>Sedum album</i>
Boraginaceae	<i>Echium vulgare</i>	Crassulaceae	<i>Sedum rupestre</i>
Geraniaceae	<i>Erodium cicutarium</i>	Asteraceae	<i>Senecio inaequidens</i>
Euphorbiaceae	<i>Euphorbia cyparissias</i>	Caryophyllaceae	<i>Silene latifolia</i>
Apiaceae	<i>Falcaria vulgaris</i>	Caryophyllaceae	<i>Silene vulgaris</i>
Lamiaceae	<i>Galiopsis angustifolia</i>	Brassicaceae	<i>Sisymbrium officinale</i>
Rubiaceae	<i>Galium mollugo</i>	Asteraceae	<i>Solidago canadensis</i>
Geraniaceae	<i>Geranium robertianum</i>	Fabaceae	<i>Trifolium pratens</i>
Asteraceae	<i>Hieracium</i> sp.	Fabaceae	<i>Trifolium repens</i>

Appendix 3. Evolutionary divergence over sequence pairs between each specimen of *H. intermedius* (calculated with Mega7.0.25).

Species 1	Species 2	Distance	Percent
Hylaeus intermedius API 006 848 {Gp 1}	Hylaeus intermedius API 006 853 {Gp 1}	0,008	0,8%
Hylaeus intermedius API 006 848 {Gp 1}	Hylaeus intermedius API 006 852 {Gp 1}	0,003	0,3%
Hylaeus intermedius API 006 853 {Gp 1}	Hylaeus intermedius API 006 852 {Gp 1}	0,002	0,2%
Hylaeus intermedius API 006 848 {Gp 1}	Barcoding Graz S25 Hylaeus int {Gp 1}	0,010	1,0%
Hylaeus intermedius API 006 853 {Gp 1}	Barcoding Graz S25 Hylaeus int {Gp 1}	0,010	1,0%
Hylaeus intermedius API 006 852 {Gp 1}	Barcoding Graz S25 Hylaeus int {Gp 1}	0,004	0,4%
Hylaeus intermedius API 006 848 {Gp 1}	Barcoding Graz S26 Hylaeus int {Gp 1}	0,005	0,5%
Hylaeus intermedius API 006 853 {Gp 1}	Barcoding Graz S26 Hylaeus int {Gp 1}	0,009	0,9%
Hylaeus intermedius API 006 852 {Gp 1}	Barcoding Graz S26 Hylaeus int {Gp 1}	0,004	0,4%
Barcoding Graz S25 Hylaeus int {Gp 1}	Barcoding Graz S26 Hylaeus int {Gp 1}	0,005	0,5%
Hylaeus intermedius API 006 848 {Gp 1}	Hylaeus intermedius KJ837822 {Gp 1}	0,002	0,2%
Hylaeus intermedius API 006 853 {Gp 1}	Hylaeus intermedius KJ837822 {Gp 1}	0,010	1,0%
Hylaeus intermedius API 006 852 {Gp 1}	Hylaeus intermedius KJ837822 {Gp 1}	0,005	0,5%
Barcoding Graz S25 Hylaeus int {Gp 1}	Hylaeus intermedius KJ837822 {Gp 1}	0,012	1,2%
Barcoding Graz S26 Hylaeus int {Gp 1}	Hylaeus intermedius KJ837822 {Gp 1}	0,007	0,7%
Hylaeus intermedius API 006 848 {Gp 1}	Hylaeus intermedius KJ838687 {Gp 2}	0,125	12,5%
Hylaeus intermedius API 006 853 {Gp 1}	Hylaeus intermedius KJ838687 {Gp 2}	0,121	12,1%
Hylaeus intermedius API 006 852 {Gp 1}	Hylaeus intermedius KJ838687 {Gp 2}	0,118	11,8%
Barcoding Graz S25 Hylaeus int {Gp 1}	Hylaeus intermedius KJ838687 {Gp 2}	0,120	12,0%
Barcoding Graz S26 Hylaeus int {Gp 1}	Hylaeus intermedius KJ838687 {Gp 2}	0,122	12,2%
Hylaeus intermedius KJ837822 {Gp 1}	Hylaeus intermedius KJ838687 {Gp 2}	0,124	12,4%
Hylaeus intermedius API 006 848 {Gp 1}	Hylaeus intermedius KJ839306 {Gp 1}	0,000	0,0%
Hylaeus intermedius API 006 853 {Gp 1}	Hylaeus intermedius KJ839306 {Gp 1}	0,007	0,7%
Hylaeus intermedius API 006 852 {Gp 1}	Hylaeus intermedius KJ839306 {Gp 1}	0,002	0,2%
Barcoding Graz S25 Hylaeus int {Gp 1}	Hylaeus intermedius KJ839306 {Gp 1}	0,007	0,7%
Barcoding Graz S26 Hylaeus int {Gp 1}	Hylaeus intermedius KJ839306 {Gp 1}	0,003	0,3%
Hylaeus intermedius KJ837822 {Gp 1}	Hylaeus intermedius KJ839306 {Gp 1}	0,002	0,2%
Hylaeus intermedius KJ838687 {Gp 2}	Hylaeus intermedius KJ839306 {Gp 1}	0,117	11,7%
Hylaeus intermedius API 006 848 {Gp 1}	Hylaeus intermedius 011 {Gp 2}	0,125	12,5%
Hylaeus intermedius API 006 853 {Gp 1}	Hylaeus intermedius 011 {Gp 2}	0,122	12,2%
Hylaeus intermedius API 006 852 {Gp 1}	Hylaeus intermedius 011 {Gp 2}	0,119	11,9%
Barcoding Graz S25 Hylaeus int {Gp 1}	Hylaeus intermedius 011 {Gp 2}	0,120	12,0%
Barcoding Graz S26 Hylaeus int {Gp 1}	Hylaeus intermedius 011 {Gp 2}	0,122	12,2%
Hylaeus intermedius KJ837822 {Gp 1}	Hylaeus intermedius 011 {Gp 2}	0,124	12,4%
Hylaeus intermedius KJ838687 {Gp 2}	Hylaeus intermedius 011 {Gp 2}	0,006	0,6%
Hylaeus intermedius KJ839306 {Gp 1}	Hylaeus intermedius 011 {Gp 2}	0,117	11,7%
Hylaeus intermedius API 006 848 {Gp 1}	Hylaeus intermedius 013 {Gp 2}	0,120	12,0%
Hylaeus intermedius API 006 853 {Gp 1}	Hylaeus intermedius 013 {Gp 2}	0,119	11,9%
Hylaeus intermedius API 006 852 {Gp 1}	Hylaeus intermedius 013 {Gp 2}	0,116	11,6%
Barcoding Graz S25 Hylaeus int {Gp 1}	Hylaeus intermedius 013 {Gp 2}	0,120	12,0%
Barcoding Graz S26 Hylaeus int {Gp 1}	Hylaeus intermedius 013 {Gp 2}	0,120	12,0%
Hylaeus intermedius KJ837822 {Gp 1}	Hylaeus intermedius 013 {Gp 2}	0,122	12,2%
Hylaeus intermedius KJ838687 {Gp 2}	Hylaeus intermedius 013 {Gp 2}	0,006	0,6%
Hylaeus intermedius KJ839306 {Gp 1}	Hylaeus intermedius 013 {Gp 2}	0,120	12,0%
Hylaeus intermedius 011 {Gp 2}	Hylaeus intermedius 013 {Gp 2}	0,000	0,0%
Hylaeus intermedius API 006 848 {Gp 1}	Hylaeus intermedius 016 {Gp 2}	0,125	12,5%
Hylaeus intermedius API 006 853 {Gp 1}	Hylaeus intermedius 016 {Gp 2}	0,122	12,2%
Hylaeus intermedius API 006 852 {Gp 1}	Hylaeus intermedius 016 {Gp 2}	0,119	11,9%
Barcoding Graz S25 Hylaeus int {Gp 1}	Hylaeus intermedius 016 {Gp 2}	0,120	12,0%
Barcoding Graz S26 Hylaeus int {Gp 1}	Hylaeus intermedius 016 {Gp 2}	0,122	12,2%
Hylaeus intermedius KJ837822 {Gp 1}	Hylaeus intermedius 016 {Gp 2}	0,124	12,4%

Hylaeus intermedius API 006 848_{Gp 1}	Hylaeus intermedius API 006 843_{Gp 2}	0,142	14,2%
Hylaeus intermedius API 006 853_{Gp 1}	Hylaeus intermedius API 006 843_{Gp 2}	0,131	13,1%
Hylaeus intermedius API 006 852_{Gp 1}	Hylaeus intermedius API 006 843_{Gp 2}	0,106	10,6%
Barcoding Graz S25 Hylaeus int_{Gp 1}	Hylaeus intermedius API 006 843_{Gp 2}	0,101	10,1%
Barcoding Graz S26 Hylaeus int_{Gp 1}	Hylaeus intermedius API 006 843_{Gp 2}	0,106	10,6%
Hylaeus intermedius KJ837822_{Gp 1}	Hylaeus intermedius API 006 843_{Gp 2}	0,108	10,8%
Hylaeus intermedius KJ838687_{Gp 2}	Hylaeus intermedius API 006 843_{Gp 2}	0,007	0,7%
Hylaeus intermedius KJ839306_{Gp 1}	Hylaeus intermedius API 006 843_{Gp 2}	0,098	9,8%
Hylaeus intermedius 011_{Gp 2}	Hylaeus intermedius API 006 843_{Gp 2}	0,000	0,0%
Hylaeus intermedius 013_{Gp 2}	Hylaeus intermedius API 006 843_{Gp 2}	0,000	0,0%
Hylaeus intermedius 016_{Gp 2}	Hylaeus intermedius API 006 843_{Gp 2}	0,000	0,0%
Hylaeus intermedius 018_{Gp 2}	Hylaeus intermedius API 006 843_{Gp 2}	0,000	0,0%
Hylaeus intermedius 024_{Gp 2}	Hylaeus intermedius API 006 843_{Gp 2}	0,000	0,0%
Hylaeus intermedius 025_{Gp 2}	Hylaeus intermedius API 006 843_{Gp 2}	0,000	0,0%
Hylaeus intermedius 014_{Gp 2}	Hylaeus intermedius API 006 843_{Gp 2}	0,013	1,3%
Hylaeus intermedius API 006 848_{Gp 1}	Hylaeus int API 006 860 gynander_{Gp 2}	0,122	12,2%
Hylaeus intermedius API 006 853_{Gp 1}	Hylaeus int API 006 860 gynander_{Gp 2}	0,118	11,8%
Hylaeus intermedius API 006 852_{Gp 1}	Hylaeus int API 006 860 gynander_{Gp 2}	0,113	11,3%
Barcoding Graz S25 Hylaeus int_{Gp 1}	Hylaeus int API 006 860 gynander_{Gp 2}	0,109	10,9%
Barcoding Graz S26 Hylaeus int_{Gp 1}	Hylaeus int API 006 860 gynander_{Gp 2}	0,114	11,4%
Hylaeus intermedius KJ837822_{Gp 1}	Hylaeus int API 006 860 gynander_{Gp 2}	0,115	11,5%
Hylaeus intermedius KJ838687_{Gp 2}	Hylaeus int API 006 860 gynander_{Gp 2}	0,006	0,6%
Hylaeus intermedius KJ839306_{Gp 1}	Hylaeus int API 006 860 gynander_{Gp 2}	0,102	10,2%
Hylaeus intermedius 011_{Gp 2}	Hylaeus int API 006 860 gynander_{Gp 2}	0,000	0,0%
Hylaeus intermedius 013_{Gp 2}	Hylaeus int API 006 860 gynander_{Gp 2}	0,000	0,0%
Hylaeus intermedius 016_{Gp 2}	Hylaeus int API 006 860 gynander_{Gp 2}	0,000	0,0%
Hylaeus intermedius 018_{Gp 2}	Hylaeus int API 006 860 gynander_{Gp 2}	0,000	0,0%
Hylaeus intermedius 024_{Gp 2}	Hylaeus int API 006 860 gynander_{Gp 2}	0,000	0,0%
Hylaeus intermedius 025_{Gp 2}	Hylaeus int API 006 860 gynander_{Gp 2}	0,000	0,0%
Hylaeus intermedius 014_{Gp 2}	Hylaeus int API 006 860 gynander_{Gp 2}	0,013	1,3%
Hylaeus intermedius API 006 843_{Gp 2}	Hylaeus int API 006 860 gynander_{Gp 2}	0,000	0,0%

Appendix 4. Pollen composition of the crop content for each investigated *Hylaeus* specimen in percent, as well as number, sampling site, date and collector of each individual.

Genus	Species	Number	Site	Date	Collector	Apiaceae	Brassicaceae	Asteraceae tubuliflorae	Asteraceae liguliflorae	Triangular/ tricolporate	Caryophyllaceae	undetermined
Hylaeus	brevicornis	7	Nbhf 1	24.08.2016	S. Schoder	44,7%	1,9%	6,7%	0,0%	46,3%	0,0%	0,3%
Hylaeus	brevicornis	8	Nbhf 2	24.08.2016	S. Schoder	49,7%	37,7%	12,7%	0,0%	0,0%	0,0%	0,0%
Hylaeus	brevicornis	9	Nbhf 2	24.08.2016	S. Schoder	1,0%	47,5%	51,2%	0,0%	0,0%	0,0%	0,3%
Hylaeus	brevicornis	10	Nbhf 1	25.08.2016	S. Schoder	0,7%	0,0%	5,6%	0,7%	92,0%	0,0%	1,0%
Hylaeus	brevicornis	15	Nbhf 2	25.08.2016	S. Schoder	53,6%	14,6%	0,0%	0,0%	30,8%	0,0%	1,0%
Hylaeus	brevicornis	20	Nbhf 2	25.08.2016	S. Schoder	5,7%	0,0%	85,0%	0,3%	9,0%	0,0%	0,0%
Hylaeus	brevicornis	21	Nbhf 1	29.08.2016	S. Schoder	98,3%	0,3%	1,0%	0,0%	0,0%	0,0%	0,3%
Hylaeus	brevicornis	706	Nbhf 1	14.09.2016	S. Schoder	18,0%	74,7%	7,3%	0,0%	0,0%	0,0%	0,0%
Hylaeus	brevicornis	524	Nbhf 4	25.08.2016	S. Schoder	33,2%	66,4%	0,3%	0,0%	0,0%	0,0%	0,7%
Hylaeus	brevicornis	161	Nbhf 4	23.06.2016	S. Schoder	82,6%	0,3%	0,0%	0,0%	16,7%	0,3%	0,0%
Hylaeus	brevicornis	250	Nbhf 1	11.07.2016	D. Zimmermann	98,3%	0,0%	0,3%	0,0%	0,0%	0,0%	1,3%
Hylaeus	brevicornis	447	Nbhf 4	04.08.2016	S. Schoder	27,7%	71,7%	0,7%	0,0%	0,0%	0,0%	0,0%
Hylaeus	brevicornis	370	Nbhf 2	09.08.2016	D. Zimmermann	93,7%	0,0%	6,0%	0,0%	0,0%	0,0%	0,3%
Hylaeus	brevicornis	359	Nbhf 1	09.08.2016	S. Schoder	99,3%	0,3%	0,3%	0,0%	0,0%	0,0%	0,0%
Hylaeus	brevicornis	31	Nbhf3	01.06.2017	S. Schoder	0,0%	11,2%	1,3%	0,0%	87,2%	0,3%	0,0%
Hylaeus	gredleri	171	Nbhf 2	23.06.2016	D. Zimmermann	97,7%	0,0%	0,0%	0,0%	2,0%	0,0%	0,3%
Hylaeus	gredleri	211	Nbhf 2	30.06.2016	S. Schoder	3,1%	0,4%	1,1%	0,0%	94,3%	0,0%	1,1%
Hylaeus	gredleri	12	Nbhf 2	09.08.2016	S. Schoder	79,0%	0,3%	20,3%	0,0%	0,0%	0,0%	0,3%
Hylaeus	gredleri	369	Nbhf 2	24.08.2016	D. Zimmermann	99,3%	0,0%	0,7%	0,0%	0,0%	0,0%	0,0%
Hylaeus	gredleri	434	Nbhf 1	04.08.2016	S. Schoder	99,3%	0,3%	0,3%	0,0%	0,0%	0,0%	0,0%
Hylaeus	gredleri	484	Nbhf 4	18.08.2016	S. Schoder	78,0%	1,0%	20,7%	0,0%	0,0%	0,0%	0,3%
Hylaeus	imparilis	19	Nbhf 1	24.08.2016	S. Schoder	3,0%	0,0%	11,0%	0,0%	86,0%	0,0%	0,0%
Hylaeus	imparilis	23	Nbhf 4	24.08.2016	S. Schoder	2,6%	70,2%	23,7%	0,0%	0,0%	0,4%	3,1%
Hylaeus	imparilis	26	Nbhf 2	25.08.2016	S. Schoder	0,0%	6,0%	94,0%	0,0%	0,0%	0,0%	0,0%
Hylaeus	imparilis	27	Nbhf 1	29.08.2016	S. Schoder	97,3%	0,0%	1,3%	0,7%	0,0%	0,3%	0,3%
Hylaeus	intermedius	24	Nbhf 4	24.08.2016	S. Schoder	0,0%	64,3%	25,1%	0,4%	0,0%	8,8%	1,3%
Hylaeus	intermedius	25	Nbhf 2	25.08.2016	S. Schoder	12,8%	7,4%	48,4%	0,0%	28,7%	0,0%	2,7%

Hylaeus	intermedius	11	Nbhf 1	24.08.2016	S. Schoder	0,3%	64,3%	35,3%	0,0%	0,0%	0,0%	0,0%
Hylaeus	intermedius	14	Nbhf 4	24.08.2016	S. Schoder	0,0%	0,0%	46,7%	0,0%	52,6%	0,0%	0,7%
Hylaeus	intermedius	16	Nbhf 4	24.08.2016	S. Schoder	0,0%	98,7%	0,7%	0,0%	0,0%	0,0%	0,7%
Hylaeus	intermedius	17	Nbhf 2	25.08.2016	S. Schoder	95,3%	3,7%	1,0%	0,0%	0,0%	0,0%	0,0%
Hylaeus	intermedius	18	Nbhf 2	25.08.2016	S. Schoder	1,0%	67,3%	31,0%	0,0%	0,0%	0,0%	0,7%
Hylaeus	intermedius	398	Nbhf 2	09.08.2016	S. Schoder	86,7%	0,0%	11,7%	0,0%	1,0%	0,3%	0,3%
Hylaeus	intermedius	360	Nbhf 1	09.08.2016	S. Schoder	99,3%	0,0%	0,3%	0,0%	0,0%	0,0%	0,3%
Hylaeus	intermedius	561	Nbhf 4	01.09.2016	S. Schoder	0,0%	77,3%	11,3%	0,0%	11,0%	0,0%	0,3%
Hylaeus	intermedius	433	Nbhf 1	04.08.2016	S. Schoder	58,3%	33,0%	4,0%	0,0%	4,0%	0,7%	0,0%
Hylaeus	intermedius	409	Nbhf 2	09.08.2016	S. Schoder	92,0%	0,0%	7,7%	0,0%	0,0%	0,3%	0,0%
Hylaeus	intermedius	28	Nbhf3	02.06.2017	S. Schoder	0,0%	0,0%	0,0%	0,0%	100,0%	0,0%	0,0%

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Jahr/Year: 2018

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