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DNA barcodes identify 99 per cent of apoid wasp species (Hymenoptera: Ampulicidae, Crabronidae, Sphecidae) from the Western Palearctic

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Abstract

The apoid wasps have traditionally been regarded as a paraphyletic assemblage of four families (Ampulicidae, Crabronidae, Heterogynidae and Sphecidae) that are closely related to the bees (Anthophila). The present study covers the three families of apoid wasps known to occur in Europe, that is, the Ampulicidae, Crabronidae and Sphecidae. DNA barcode sequences of 3,695 specimens of apoid wasps were analysed for the present study, including 21 specimens of Ampulicidae, 3,398 Crabronidae and 276 Sphecidae. The sequences of the dataset represent 661 species of apoid wasps, including two species of Ampulicidae, 613 of Crabronidae and 46 species of Sphecidae. The dataset includes DNA barcodes of 240 species of German apoid wasps, representing 88% of the German fauna, and 578 European species, representing 65% of the European apoid wasp fauna. The study demonstrates that virtually all species of the three examined families can be reliably identified by DNA barcodes. The implications of highly congruent results between traditional taxonomy and DNA barcoding for the reliable application of DNA-based identifications are discussed.

1 | INTRODUCTION

The present study provides the first attempt to compile a comprehensive DNA barcode library for apoid wasps, called also digger wasps, Spheciformes or “sphecids,” for the Western Palearctic region. Apoid wasps are traditionally classified in four families, Ampulicidae, Crabronidae, Heterogynidae and Sphecidae. Although recent research suggests a new classification based on a phylogenomic analysis of the Apoidea with ten separate families (Sann et al., 2018), the present study uses the traditional classification with four families of apoid wasps, all except the Heterogynidae are dealt with here. Together with the bees (Anthophila, consisting of seven families), they are usually subsumed under the superfamily Apoidea and belong to the megadiverse insect order Hymenoptera with currently

more than 155,000 described species (Aguilar et al., 2013). Worldwide, about 9,900 species of apoid wasps have been described (Pulawski, 2018). Although there are no published species numbers for the Western Palearctic region, the number of species is estimated at about 1,500 (Schmid-Egger, unpublished). In Europe, about 730 species have been recorded (Barbier, 2013), and 273 species are known from Germany (Jacobs, 2007, CSE, unpublished).

The initial phase of the DNA barcoding projects focused on species occurring in southern Germany (Bavaria), as part of the “Barcoding Fauna Bavarica” project of the SNSB-Zoologische Staatssammlung in Munich, Germany (ZSM). The project commenced in 2009 and aimed at assembling DNA barcodes for all Bavarian animal species (Hausmann et al., 2013, 2012; Hendrich et al., 2010).

Since 2012, the "German Barcode of Life" (GBOL) project adds additional sequences, and in 2015, the German projects were merged with the DNA barcoding projects of the Institute of Biodiversity and Ecosystem Research (formerly Institute of Zoology of the Bulgarian Academy of Sciences).

The barcoding projects were conducted in close cooperation with the Center for Biodiversity Genomics, University of Guelph, within the framework of the International Barcode of Life (iBOL) initiative. All sequences and the associated project data are available through the Barcode of Life Data Systems (BOLD, www.boldsystems.org). Although the dataset includes records from an area ranging from the eastern to the western border of the Palearctic region, most records are from the central and southern parts of the Western Palearctics where the majority of species occurs.

2 | MATERIALS AND METHODS

2.1 | Sampling

The present study covers the three families of apoid wasps known to occur in the Western Palearctic region, that is, Ampulicidae, Crabronidae and Sphecidae (not counting the Heterogynaidae with a few rare species). The main source of specimens includes (a) specimens from Central Europe: from the collections of the Zoologische Staatssammlung München (ZSM), the private collection of Christian Schmid-Egger (CSE) and the private collection of Jakub Straka (JS), (b) southern Europe and Turkey: from the collection of the Institute of Biodiversity and Ecosystem Research (mainly from Turkey, Bulgaria and Central Italy (Abruzzo)), (c) United Arab Emirates and Morocco: from the private collection of CSE and (d) a large portion of *Tachysphex* specimens from the private collection of JS. For additional material from colleagues, see the Acknowledgments section. Some specimens of species recorded from Germany were not collected in Germany but in neighbouring countries, mainly in northern Italy, the Czech Republic and Hungary because these species are rare or even close to extinction in Germany and therefore virtually impossible to obtain from German territory.

For DNA extraction, a single leg was removed from each adult specimen and sent to the Canadian Centre for DNA Barcoding (CCDB) in Guelph, Canada, for DNA extraction and barcode sequencing. Specimens were identified to species level using the most recent literature, including Jacobs (2007) for Germany and Bitsch et al. (2007) for Europe (see also Schmid-Egger, 2011, 2014 for species of the UAE). A complete list of voucher specimens included in the current release is given in Appendix S1.

2.2 | DNA sequencing

DNA extraction, PCR amplification and sequencing were conducted at the Canadian Centre for DNA Barcoding (CCDB) using standardized high-throughput protocols (deWaard, Ivanova, Hajibabaei, & Hebert, 2008; Ivanova, deWaard, & Hebert, 2006; <http://www.ibolproject.org/resources.php>). The 658-bp target region, starting from

the 5' end of the mitochondrial cytochrome c oxidase I (COI) gene, includes the DNA barcode region of the animal kingdom (Hebert, Cywinska, Ball, & deWaard, 2003). The DNA extracts are stored at the CCDB with aliquots being deposited at the DNA-Bank facility at the ZSM, as part of the DNA-Bank Network (see www.dnabank-network.org). Specimens that were successfully sequenced are listed in Appendix S1, with sequence lengths and the number of unresolved bases. All specimen data are accessible in BOLD as a single citable dataset (<https://doi.org/10.5883/DS-SPHECEU>). The data include collecting locality, geographic coordinates, elevation, collector, one or more digital images, identifier and voucher depository. Sequences data can be obtained through BOLD, and they include a detailed LIMS report, primer information and access to trace files. The sequences are also available on GenBank (Accession nos. MH608369–MH611354).

2.3 | Data analysis

Sequence divergence statistics were calculated using the Kimura 2-parameter model of sequence evolution (Kimura, 1980). Barcode Index Numbers (BINs) were assigned by the BOLD system, representing globally unique identifiers for clusters of sequences that correspond closely to biological species (Ratnasingham & Hebert, 2013). For BIN assignment, a minimum sequence length of 500 bp is required, and sequences between 300 and 500 bp can join an existing BIN but will not create or split BINs. BINs provide an interim taxonomic system and a way to signify molecular operational taxonomic units (MOTUs) prior to detailed taxonomic studies including morphology. Sequences were aligned using the BOLD Aligner (amino acid-based hidden Markov models). The analyses are based on sequences with a minimum length of 500 bp and <1% ambiguous bases. Genetic distances and summary statistics were calculated using analytical tools in BOLD and are given as mean and maximum pairwise distances for intraspecific variation and as minimum pairwise distances for interspecific variations. Haplotypes were inferred using DnaSP 6 (Rozas et al., 2017), and TCS networks (Clement, Snell, Walker, Posada, & Crandall, 2002) constructed and drawn using POPART version 1.7 (Leigh, Bryant, & Nakagawa, 2015).

3 | RESULTS

For the present study, DNA barcode sequences of 3,695 specimens of apoid wasps were analysed, including 21 specimens of Ampulicidae, 3,398 Crabronidae and 276 Sphecidae. Of those sequences, 3,628 had a length of at least 500 bp and <1% ambiguous bases and were used for further analyses. The sequences of the dataset represented 661 species, including two species of Ampulicidae, 613 of Crabronidae and 46 species of Sphecidae (Table 1). The dataset includes DNA barcodes of 240 species of German apoid wasps, representing 88% of the German fauna, and, with 578 European species, 65% of the European apoid wasp fauna (Table 1).

The highest number of specimens per country was collected in Germany (1,432 specimens), followed by Italy (511 specimens) and

TABLE 1 Number of German and European species of Ampulicidae, Crabronidae, and Sphecidae, and absolute and relative numbers of species included in the current release for Germany and Europe. Data for Europe are based on the Fauna Europaea (Barbier, 2013)

	Species in Germany	German species in release	Species in Europe	European species in release	Total species in release
Ampulicidae	3	1 (33%)	5	1 (20%)	2
Crabronidae	258	228 (88%)	816	527 (65%)	613
Sphecidae	12	11 (92%)	72	50 (69%)	46
Total	273	240 (88%)	893	578 (65%)	661

Bulgaria (346 specimens). These three countries plus Morocco, United Arab Emirates, Greece and Turkey account for 81% of the specimens (Figure 1; Table 2).

The 3,628 sequences used for further analysis included 655 species, with the same number of Ampulicidae and Sphecidae (2 and 46, resp.), but only 607 species of Crabronidae instead of 613 species in the complete dataset. The largest genetic distances within each family ranged from 26.4% in the Ampulicidae to 45.8% in the Crabronidae (Table 2). The mean within-species distances across the three families ranged from 1.6% in the Crabronidae to 2.0% in the Sphecidae and 2.8% in the Ampulicidae (Table 3).

Sharing of the same Barcode Index Number (BIN) between two or more species was observed in 17 species, all of them Crabronidae. Whereas twelve species with BIN sharing could be identified

TABLE 2 Number of species and specimens for countries with ten or more species. Eleven more countries are represented in the dataset by less than five species each

Country	Species	Specimens
Germany	200	1,432
Bulgaria	138	346
Italy	125	511
United Arab Emirates	115	216
Morocco	95	218
Hungary	85	125
Turkey	76	133
Czech Republic	53	99
France	50	90
Cyprus	40	83
Greece	34	137
Spain	28	66
Iran	26	30
Tunisia	21	29
Croatia	18	32
Austria	14	23
Oman	12	15
Mongolia	12	13
Jordan	11	12
Portugal	10	17
Poland	10	16
Israel	10	12
Slovakia	10	11

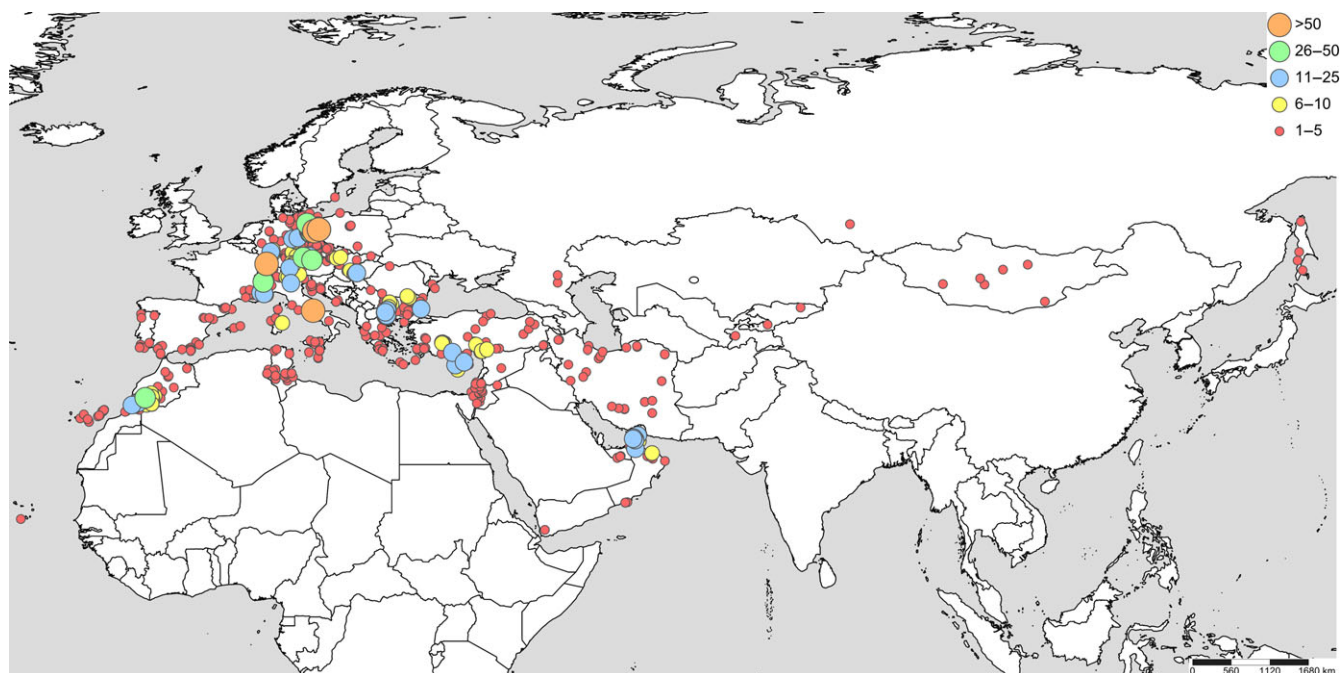


FIGURE 1 Collecting sites of 3,628 specimens of Ampulicidae, Crabronidae and Sphecidae in the Palearctic region, with dot size and circle colour indicating the number of specimens collected at each site

TABLE 3 Genetic distances (K2P model) within species, genus, and family for the Ampulicidae, Crabronidae, and Sphecidae. For each taxon, the number of taxa, minimum, maximum and average distances are given in percent

	Ampulicidae				Crabronidae				Sphecidae			
	Taxa	Min	Max	Avg	Taxa	Min	Max	Avg	Taxa	Min	Max	Avg
Species	2	0.00	8.68	2.79	411	0.00	18.23	1.58	75	0.00	17.66	1.99
Genus	1	16.54	16.54	16.54	53	0.00	24.22	11.98	11	0.00	26.33	14.85
Family	1	20.72	26.44	23.33	1	6.88	45.79	24.21	1	10.35	35.73	21.43

using a tree-based approach (i.e., they belong to different subclusters within the same BIN), the reliable identification of five species in two species groups (*Pemphredon enslini* (Wagner, 1932)—*P. lethifer* (Shuckard, 1837)—*P. littoralis* (A. Wagner, 1918) and *Pseudoscolia camela* (Schmid-Egger, 2014)—*P. dewitzi* (Kohl, 1889)) was problematic using DNA barcodes, equalling one per cent of all species included in the present study. Both species groups belong to taxonomically challenging species complexes and results of DNA barcoding need to be critically re-evaluated as part of an in-depth taxonomic treatment.

3.1 | Haplotype divergence

The presence of two or more BINs in one species occurred in a comparatively large number of species. Excluding 27 species with BIN divergence (i.e., multiple BINs within a species) that belong to the crabronid genus *Tachysphex* (Crabronidae), a group that is currently under revision (JS Straka, in prep.) and that contains many

species with interim names awaiting formal description, 104 species exhibit BIN divergence (Table 4). Most species with BIN divergence include two or three BINs, and only a few species contain four, six or seven BINs. This high level of BIN divergence could even be observed in 69 species from Germany, a country that has one of the best-known faunas of apoid wasps in the world.

Populations with species that exhibited BIN divergence may occur sympatrically or allopatrically. In 40 species (38%) with BIN divergence, populations with different BINs occur allopatrically. These populations may either represent a widespread and genetically variable species (i.e., *Harpactus laevis* (Latreille, 1792), *H. pulchellus* (A. Costa, 1859)), or they may belong to distinct species that were previously overlooked as in *Tachysphex pompiliformis* (Panzer, 1804) (Straka, 2016). In 34 species (33%), the BIN divergence occurred in sympatric populations, indicating the presence of species complexes. The other species with BIN divergence showed both populations with allopatric and sympatric distribution. Species with a wider distribution in the Mediterranean area showed several different spatial

TABLE 4 Species with BIN divergence, with species grouped by the number of BINs present in each species, for each of the three families Ampulicidae (A), Crabronidae (C), and Sphecidae (S). (Species of *Tachysphex* excluded, see text for further explanation)

BINs	Species
A 4	<i>Dolichurus corniculus</i>
C 2	<i>Alysson spinosus</i> , <i>Astata boops</i> , <i>Astata costae</i> , <i>Astata minor</i> , <i>Belomicrus italicus</i> , <i>Bembecinus hungaricus</i> , <i>Bembecinus peregrinus</i> , <i>Bembix bolivari</i> , <i>Bembix olivacea</i> , <i>Cerceris quadricincta</i> , <i>Cerceris quadrifasciata</i> , <i>Cerceris sabulosa</i> , <i>Cerceris stratiotes</i> , <i>Crossocerus leucostoma</i> , <i>Crossocerus pullulus</i> , <i>Dinetus dentipes</i> , <i>Dinetus pictus</i> , <i>Diodontus minutus</i> , <i>Diodontus tristis</i> , <i>Dryudella stigma</i> , <i>Ectemnius borealis</i> , <i>Ectemnius continuus</i> , <i>Ectemnius crassicornis</i> , <i>Ectemnius fossorius</i> , <i>Ectemnius lapidarius</i> , <i>Ectemnius lituratus</i> , <i>Entomognathus brevis</i> , <i>Gastrosericus moricei</i> , <i>Gastrosericus waltlii</i> , <i>Harpactus elegans</i> , <i>Harpactus formosus</i> , <i>Harpactus niger</i> , <i>Harpactus pollux</i> , <i>Harpactus tumidus</i> , <i>Hoplisoides punctuosus</i> , <i>Lindenius albilabris</i> , <i>Lindenius laevis</i> , <i>Lindenius melinopus</i> , <i>Liris agilis</i> , <i>Liris haemorrhoidalis</i> , <i>Liris niger</i> , <i>Liris subtessellatus</i> , <i>Mimesa lutaria</i> , <i>Miscophus ater</i> , <i>Miscophus dispersus</i> , <i>Miscophus eatoni</i> , <i>Miscophus spurius</i> , <i>Nitela truncata</i> , <i>Nysson dimidiatus</i> , <i>Nysson maculosus</i> , <i>Oxybelus bipunctatus</i> , <i>Oxybelus cocacola</i> , <i>Oxybelus lamellatus</i> , <i>Oxybelus quatuordecimnotatus</i> , <i>Oxybelus uniglumis</i> , <i>Passaloecus borealis</i> , <i>Passaloecus gracilis</i> , <i>Passaloecus singularis</i> , <i>Pemphredon enslini</i> , <i>Pemphredon lugens</i> , <i>Pemphredon lugubris</i> , <i>Pemphredon wesmaeli</i> , <i>Philanthus variegatus</i> , <i>Pison atrum</i> , <i>Prosopigastra handlirschi</i> , <i>Psenulus pallipes</i> , <i>Pseudoscolia lyauteyi</i> , <i>Spilomena beata</i> , <i>Spilomena troglodytes</i> , <i>Stigmus solskyi</i> , <i>Stizus kohlii</i> , <i>Trypoxylon aegyptius</i> , <i>Trypoxylon deceptorium</i> , <i>Trypoxylon medium</i> , <i>Trypoxylon minus</i> , <i>Trypoxylon scutatum</i>
3	<i>Bembecinus tridens</i> , <i>Crossocerus quadrimaculatus</i> , <i>Gorytes sulcifrons</i> , <i>Harpactus laevis</i> , <i>Harpactus pulchellus</i> , <i>Harpactus transiens</i> , <i>Lestica clypeata</i> , <i>Miscophus niger</i> , <i>Nysson dimidiatus</i> , <i>Oxybelus mucronatus</i> , <i>Pemphredon lethifer</i> , <i>Philanthus coarctatus</i> , <i>Philanthus triangulum</i> , <i>Prosopigastra creon</i> , <i>Solierella compedita</i>
4	<i>Harpactus lunatus</i>
6	<i>Harpactus affinis</i> , <i>Miscophus helveticus</i>
7	<i>Bembix oculata</i> , <i>Diodontus insidiosus</i>
S 2	<i>Ammophila campestris</i> , <i>Ammophila pubescens</i> , <i>Podalonia alpina</i> , <i>Podalonia tydei</i> , <i>Prionyx lividocinctus</i> , <i>Sceliphron destillatorium</i> , <i>Sphex funerarius</i> , <i>Sphex pruinosus</i>
3	<i>Ammophila heydeni</i> , <i>Palmodus occitanicus</i> , <i>Prionyx pollens</i>
5	<i>Podalonia hirsuta</i>

patterns of BIN sharing and/or BIN divergence, as exemplified below.

Species with different BINs in allopatric populations include *Ammophila heydeni* Dahlbom, 1845, *Bembix oculata* Panzer, 1801 and *B. flavescens* F. Smith, 1856. In these and other apoid wasps, variation in morphology and colour pattern of specimens from different localities had been assigned little importance by taxonomists, and species were usually interpreted in a wide sense. In *A. heydeni*, de Beaumont (1967) suggested the existence of more than one species based on colour pattern and morphology. DNA barcoding of specimens from Bulgaria, Cyprus, Czech Republic, Hungary and Italy resulted in three BINs that possibly represent different species, and existence of additional species in areas where the species occurs like Turkey or Sardinia is highly probable.

Each of the two species *Bembix oculata* and *B. flavescens* have traditionally been treated either as two distinct species with several subspecies, or as two complexes of different species. Whereas *B. oculata* included several, albeit not widely accepted subspecies (see Pulawski, 2018 for details), *B. flavescens* was treated as several distinct species, or species with subspecies that were widely accepted. De Beaumont (1957) synonymized all taxa under *B. flavescens* (with exception of *B. turca* Dahlbom, 1845 from Turkey that he kept as separate species). DNA barcoding results show that each of the two species lineages contains several distinct subclusters (Appendix S3) with haplotypes showing an allopatric distribution. In *B. flavescens*, the subclusters are in perfect agreement with de Beaumont's (1957) subspecies, with genetic distances ranging from 2.8% to 6.6% (Appendix S2), and we suggest treating the subclusters as distinct species, that is, *B. flavescens* (endemic to Canary Islands), *Bembix bolivari* Handlirsch, 1893 (Portugal and Sicily), *B. citrina* Mercet, 1905 (southwestern Morocco) and *B. kittya* de Beaumont, 1957 (Tunisia). Other taxa in this complex were not examined, but it is to be expected that at least *B. flavescens picturata* Bytinski-Salz, 1955 from Israel represents a distinct species. It is noteworthy that *B. bolivari* from Sicily differs markedly from the specimens from Portugal and both may belong to different species. *Bembix turca* with specimens from Turkey and Bulgaria examined came out close to the *B. flavescens* species complex. *Bembix oculata* shows a pattern similar to *B. flavescens* of genetic variation and geographic distribution (Figure 2). The species is morphologically as uniform as *B. flavescens* but exhibits high levels of colour variation that do not match to geographic origin (except specimens from Cyprus that differ markedly by colour).

The European beewolf, *Philanthus triangulum* (Fabricius, 1775), contains individual haplotypes that occur in several countries including Bulgaria, Cyprus, Turkey and the UAE, and a common haplotype that is widely distributed and occurs in several Central European and Mediterranean countries (Figure 2). This haplotype includes specimens from Morocco that were regarded to represent a distinct species based on colour differences (Pulawski, 2018). The maximum intraspecific distance of 1.9% (Appendix S2) does not suggest the existence of more than one species.

Lestica clypeata (Schreber, 1759) exhibits a high level of genetic divergence with 21 haplotypes being distributed in three groups, each with a separate BIN (Appendix S2), with ten haplotypes distributed in Bulgaria, Germany and Italy, nine haplotypes in Bulgaria, Croatia, Cyprus and Italy, and two haplotypes in Morocco (Figure 2). The high intraspecific distance of 7.8% suggests the existence of several species. Noteworthy is the cluster of similar haplotypes that occurs mainly in Germany and Italy, with multiple connective links (H01, H03–H05, H12–H16), and the occurrence of a second major cluster of haplotypes that occurs mainly in Italy (H06, H08–H11) indicating a scenario of geographic separation and subsequent spread of populations from southern Europe northwards.

3.2 | The genetic gap between Europe and North Africa

In 31 species with BIN divergence, genetically different populations are divided into two clusters with populations from northwest Africa (Morocco and Tunisia) in one cluster and European and/or western Asian populations in a separate cluster. Whereas some of the North African taxa had already been recognized as distinct species that are different from Eurasian populations, most cases are still in need of critical examination.

3.3 | The fauna of Cyprus

Cyprus is known for its distinct fauna of apoid wasps with numerous endemic species (Balthasar, 1954; de Beaumont, 1947). In several species, DNA barcoding resulted in genetic distances between specimens from Cyprus and from mainland populations (e.g., *Dolichurus corniculatus* (Spinola, 1807), *Gastrosericus waltlii* (Spinola, 1839), *Mischophus niger* (Dahlbom, 1844), *Podalonia hirsuta* (Scopoli, 1763), Appendix S2), some of them paralleled by distinct morphological differences and treated by taxonomists as subspecies, (*Ammophila heydeni* (see de Beaumont, 1957), *Mischophus mavromoustakisi* de Andrade, 1953, *Tachysphex helveticus* Kohl, 1885), as separate species (*Mischophus helveticus* Kohl, 1883 (see de Andrade, 1954)), or still in need of taxonomic revision (*Belomicrus italicus* A. Costa, 1867).

4 | DISCUSSION

The present release demonstrates that virtually all species of apoid wasps (Ampulicidae, Crabronidae and Sphecidae) from the West Palearctic that were examined in our study can be reliably identified by DNA barcodes. Only in five species representing two species complexes, the DNA-based assignment to species is problematic, but in these cases, preliminary evidence suggests that the apparent barcoding failure will disappear once a detailed taxonomic treatment of the taxa in question has been conducted (CSE, in prep.).

A comparison of DNA barcoding results between German species of apoid wasps and bees (Anthophila) reveals similar degrees of

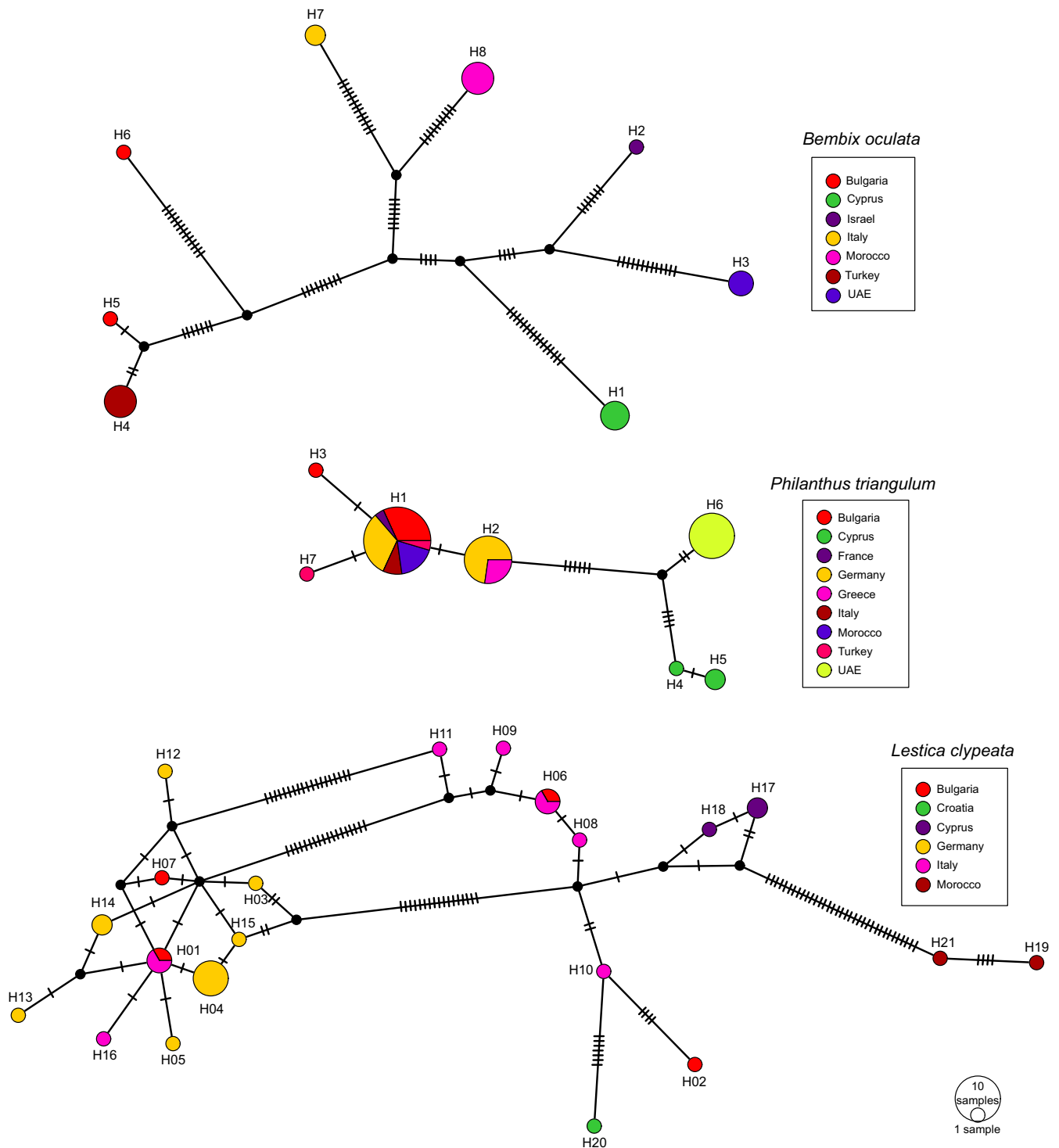


FIGURE 2 Haplotype networks of three species of *Bembyx oculata*, *Philanthus triangulum* and *Lestica clypeata* (Crabronidae)

species with BIN discordance, although the percentage of problematic species is lower in apoid wasps (5 species, 0.8%) compared to the bees (15 species, 2.6%, see Schmidt, Schmid-Egger, Morinière, Haszprunar, & Hebert, 2015). Similar outcomes of DNA barcoding were to be expected since bees are phylogenetically deeply nested within apoid wasps (Sann et al., 2018).

4.1 | DNA barcoding of German species

For the present study, 228 of the 240, or about 88%, of the apoid wasps that are known to occur in Germany were analysed by DNA barcoding. This is similar to a recent DNA barcoding study dealing with German bees (Schmidt et al., 2015), in which the same

percentage of German species was barcoded, although the number of German species is, with 584 species (Scheuchl & Schwenninger, 2015), more than twice as large as the number of apoid wasp species. Despite similar fractions of the German fauna of bees and apoid wasps being included, percentages of species that exhibit BIN discordance vary between the two groups of Aculeata. Bees show an approximately two times higher relative amount of BIN sharers, species with BIN divergence and taxonomically problematic species that cannot be reliably identified by DNA barcodes. Furthermore, there are significantly more taxonomically problematic taxa of bees that are controversially discussed among taxonomists compared to species of apoid wasps. This is probably partly due to the observation that problematic bee taxa receive more attention by taxonomists and are more often controversially discussed than apoid wasps (see Schmidt et al., 2015 for discussion). Another factor that could be significant is the younger age of bees, a group that evolved during the last 130 Million years, and their strong radiation during this time, compared to the major lineages of apoid wasps that are older and less diverse (Sann et al., 2018). The putative sister group of the bees sensu the new classification, Ammoplanidae (=Ammoplanina sensu Bohart & Menke, 1976), includes <150 species in 10 genera (Pulawski, 2018), representing a comparatively low number compared to the more than 20,000 species of bees (Ascher & Pickering, 2018).

4.2 | BIN sharing

BIN sharing was found in several species and although these species could be identified using a tree-based approach, the underlying problems are different in each of the affected taxa, and in several cases aided in resolving long-standing taxonomic problems, as exemplified below.

Oxybelus haemorrhoidalis Olivier, 1812 and *O. victor* Lepeletier de Saint Fargeau, 1845 were treated as conspecific (Jacobs, 2007) or the latter was treated as a subspecies of *O. haemorrhoidalis* (Bitsch et al., 2007). A low intraspecific distance of 1.3% (Appendix S2) between specimens from Bulgaria, Czech Republic, Germany and Hungary supports the conspecificity of *O. haemorrhoidalis* and *O. victor*, although more and reliably identified material of *O. victor* needs to be examined.

Morphological separation of *Trypoxylon clavicerum* Lepeletier de Saint Fargeau and Audinet-Serville, 1828 and *T. kostylevi* Antropov, 1985 has been most challenging or even impossible (Antropov, 1985; Jacobs, 2007) because characters in the female are subject to variation and because differences in male genitalia character may be caused by preparation (Jacobs, Doczkal, pers. comm., CSE, TL pers. obs.). DNA barcoding of 35 specimens from both putative species from four different countries resulted in intraspecific distances of up to 2.8% but search for diagnostic morphological characters did not reveal any reliable characters for separation (CSE, unpublished), suggesting the presence of a single species.

Crossocerus elongatulus (Vander Linden, 1829), *C. italicus* (not recorded from Germany) and *C. distinguendus* (A. Morawitz, 1866) have traditionally been recognized as distinct species (Jacobs &

Schmid-Egger, 2014) despite their BIN sharing. However, the three species could be identified using a tree-based approach.

Lestica subterranea (Fabricius, 1775) and *L. alata* (Panzer, 1797) are morphologically distinct (Jacobs, 2007) but despite the two species showing BIN sharing (Appendix S2), they are identifiable using tree-based identification.

Finally, *Pemphredon lethifer* and *P. littoralis* have been controversial (see Jacobs, 2007 for details). DNA barcoding of 36 specimens from nine countries, four of them identified as *P. littoralis*, did not reveal any substantial differences between the two species. Moreover, it appears that specimens of *P. lethifer* from several Central European and Mediterranean countries were represented by a separate BIN (Appendix S2). The *P. lethifer* group is morphologically and genetically very similar and either represents a single variable species, or multiple very similar and variable species as it has been recently recognized in *Tachysphex* (Straka, 2016) and *Pemphredon* (Smissen, 2003). This leaves two species of the German fauna that cannot be reliably identified using DNA barcodes.

4.3 | DNA barcoding and traditional taxonomy

The present dataset provides a comparatively high coverage of the Central European fauna of apoid wasps. Because of their attractive appearance, they have received ample attention by taxonomists. This allows some insights into the performance of traditional taxonomic approaches using morphology and results through DNA barcoding. As with most other insects, traditional taxonomy in apoid wasps has been primarily based on examination of differences in morphology and colour. Only occasionally, other characters like phenology, behaviour and ecology of geographic distribution patterns have received attention, and at least in Germany, not a single species has ever been recognized on any of the latter characters in the absence of morphological differences. The evaluation of morphological differences between species, in particular if species are similar with subtle differences, is affected by a number of factors, including experience of the researcher, optics, lighting, and availability of sufficient material for comparison. For example, characters like the degree to which the frons is bulged in a species can only be assessed and described in direct comparison with other species, and illustrations, even if high-quality, do often not replace the need for examination of physical specimens. Factors that can affect the morphological assessment change over the time, leading to different concepts that are reflected in the literature that deals with the taxonomy of a particular taxon. This is caused by irreproducible assessment of morphological characters by different taxonomists at different points in time, in particular when dealing with allopatric populations where species delimitations are to a large degree subjective and to some extent dependent on the applied species concept (Mutanen et al., 2012).

A high level of congruence between traditional taxonomy and DNA barcoding not only puts trust in previous taxonomic treatments of the group, but also it allows for reliable application of DNA-based identification. Furthermore, it also will alleviate the need for comparing and discussing different taxonomic concepts that accumulated

over time and that are often influenced by opinion and personal preferences of taxonomists, thus leading to an increased objectivity of taxonomy (Kekkonen & Hebert, 2014). Species that exhibit apparent barcode failure need to be scrutinized and DNA barcodes may not provide enough resolution in which case supplementary markers seem necessary, although close examination of cases of incongruence may reveal that they are often caused by operational factors like misidentifications, oversplitting or overlooked species, species delimitation issues or other methodological errors (Mutanen et al., 2016).

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DATA ACCESSIBILITY

All specimen data are accessible on BOLD (www.boldsystems.org) through the following link <https://doi.org/10.5883/DS-SPHECEU>. The data include collection locality, geographic coordinates, altitude, collector, one or more images, identifier and voucher depository. Sequence data are available on BOLD and include a detailed LIMS report, primer information and trace files, and sequences have been deposited also in GenBank (Accession nos. MH608369–MH611354).

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