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DNA barcoding largely supports 250 years of classical taxonomy: identifications for Central European bees (Hymenoptera, Apoidea *partim*)

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Abstract

This study presents DNA barcode records for 4118 specimens representing 561 species of bees belonging to the six families of Apoidea (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae and Melittidae) found in Central Europe. These records provide fully compliant barcode sequences for 503 of the 571 bee species in the German fauna and partial sequences for 43 more. The barcode results are largely congruent with traditional taxonomy as only five closely allied pairs of species could not be discriminated by barcodes. As well, 90% of the species possessed sufficiently deep sequence divergence to be assigned to a different Barcode Index Number (BIN). In fact, 56 species (11%) were assigned to two or more BINs reflecting the high levels of intraspecific divergence among their component specimens. Fifty other species (9.7%) shared the same Barcode Index Number with one or more species, but most of these species belonged to a distinct barcode cluster within a particular BIN. The barcode data contributed to clarifying the status of nearly half the examined taxonomically problematic species of bees in the German fauna. Based on these results, the role of DNA barcoding as a tool for current and future taxonomic work is discussed.

Keywords: bees, DNA barcoding, insects, morphology, pollinators, taxonomy

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Introduction

Much global food crop production depends, directly or indirectly, on pollination by insects with bees playing a particularly important role in both managed and natural ecosystems (e.g. Williams 1994; Roubik 1995; Klein *et al.* 2007). While there is increasing evidence that the service provided by honeybees is at risk (e.g. Palmer *et al.* 2004; Burkle *et al.* 2013), wild bees can compensate for this loss of pollination services. Garibaldi *et al.* (2013) found that wild insects actually pollinated crops more efficiently, were more consistent in causing fruit production, and that these effects on pollination were independent of the presence of honeybees. With honeybees in global decline and wild bees threatened by habitat fragmentation and degradation (e.g. Potts *et al.* 2010; Vanbergen and the Insect Pollinators Initiative 2013), there is an increasing demand for the reliable identification of species of wild bees.

This study represents the first step in the development of a complete DNA barcode library for German species of bees and wasps (Hymenoptera: Aculeata). The initial phase of this project targeted Bavarian species as it was part of the Barcoding Fauna Bavarica project, led by the Zoologische Staatssammlung in Munich (ZSM), which seeks to assemble DNA barcodes for all animal species in this state (Hendrich *et al.* 2010; Hausmann *et al.* 2012, 2013a,b). Within 5 years, about 45 000 barcode sequences representing over 12 000 species were generated, including records for almost 2500 species of Hymenoptera. Additional samples were acquired through the German Barcode of Life (GBOL) project that commenced in 2012. Both projects were conducted in close cooperation with the Biodiversity Institute of Ontario within the framework of the International Barcode of Life (iBOL) project. All sequences and the associated specimen data are available through the Barcode of Life Database (BOLD, www.boldsystems.org).

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Materials and methods

Sampling

Between 2009 and 2014, 6112 specimens from the collections of the ZSM and the private collection of CS-E and

other specialists representing about 600 species of bees were processed. Because of the geographic position and size of the study area, a large fraction of the species occurring in Central Europe is included in this study. Because there is no recent checklist, we employed an unpublished checklist of German bees by Schmid-Egger and obtained distributional information and nomenclature from Kuhlmann *et al.* (2014). Specimens were identified using the most recent literature for each taxon (Amiet 1996; Scheuchl 1995, 2006; Schmid-Egger & Scheuchl 1997; Amiet *et al.* 1999, 2001, 2004, 2007, 2010) . Appendix S1 (Supporting information) provides a complete list of voucher specimens with sample ID, country of origin, Barcode Index Number and GenBank Accession no. All specimens are deposited in the ZSM Hymenoptera section.

Despite a strong emphasis on specimens collected from Bavaria in south-east Germany, specimens from other localities were used to extend species coverage. Most of these specimens derived from Germany, but some were sourced from adjacent areas, especially the Southern Alps, if no recent German material was available. A few species included in this release have not yet been recorded from Germany, but they may be present because of their occurrence in neighbouring countries.

DNA sequencing

A single leg from each specimen was submitted to the Canadian Centre for DNA Barcoding (CCDB) for sequence analysis. DNA extraction, PCR amplification and sequencing were conducted using standardized high-throughput protocols (Ivanova *et al.* 2006; deWaard *et al.* 2008, http://www.ccdb.ca/resources.php). The 658 bp target region, near the 5′ terminus of the mitochondrial cytochrome *c* oxidase (COI) gene, includes the DNA barcode region for the animal kingdom (Hebert *et al.* 2003). The DNA extracts are stored at both the CCDB and the DNA-bank facility at the ZSM as part of the DNA Bank Network (www.dnabank-network.org). Following sequence analysis, the records were divided into two projects, one (GBAPI) containing all barcode compliant (>500 bp) records and the other (GBAPS) containing shorter sequences. All specimen data are accessible on BOLD through the following DOIs: dx.doi.org/10.5883/DS-GBAPI and dx.doi.org/10.5883/DS-GBAPS. The data include collection locality, geographic coordinates, altitude, collector, one or more images, identifier and voucher depository. Sequence data are available on BOLD including a detailed Laboratory Information Management System (LIMS) report, primer information and trace files and also on GenBank (Accession nos. KJ836402–KJ839833, Appendix S1, Supporting information).

Data analysis

Analysis was restricted to the subset of sequences which met barcode standards (sequence length >500 bp, <1% ambiguous bases, bidirectional sequencing, country specification). Sequences were aligned using the BOLD Aligner and divergences were calculated using the Kimura 2-parameter (K2P) distance model (Kimura 1980) using the analytical tools in BOLD. The results are reported as mean and maximum pairwise distances for intraspecific variation and as minimum pairwise distance for interspecific variation. Barcode compliance requires that sequences meet certain quality standards, and it has the secondary benefit of ensuring that each record is assigned a Barcode Index Number (BIN) by BOLD (Ratnasingham & Hebert 2013). A globally unique identifier (i.e. BIN) is assigned to each sequence cluster, creating an interim taxonomic system because the members of a particular BIN often correspond to a biological species. The BIN system provides an automated way to delineate molecular operational taxonomic units (MOTUs) in a structured way that provides a useful basis for subsequent detailed taxonomic studies including morphology.

Results

Sequence recovery

COI sequences were recovered from 4118 of the 6112 specimens that were analysed with barcode compliant records from 3183 specimens representing 514 species (Table 1). Sixty-eight German species were not available for barcode analysis (Appendix S2, Supporting information). Shorter sequences were recovered from 935 specimens representing 363 species, most of which also had full-length records. However, 47 species (including 43 species in the German fauna) were only represented by short sequences (Appendix S3, Supporting information, Table 1). Full-length barcodes were not recovered from several species with fresh material (e.g. *Andrena flavipes* – 17 specimens, *Camptopoeum frontale* and *Chelostoma florisomne* – seven specimens each, *Andrena curvungula* – six specimens, Appendix S3, Supporting information). Despite these problematic taxa, this study generated 4118 sequences that provide at least a partial barcode sequence for 561 species (Table 1). Sequence divergence ranged from 0 to 12.2% with a mean distance of 0.58% within species, and from 0 to 29.3% with a mean of 14.1% within genus (Fig. 1).

BIN sharing and BIN divergence

The following five sections deal with species that exhibit BIN divergence, BIN sharing or both. Species with BIN divergence are treated under three categories: (1) species

Table 1 Number of bee specimens with barcode records >500 bp or <500 bp and the number of species represented from Germany and from all European localities examined in this study

Sequence length	Specimens	All species	German species
>500 bp	3183	514	503
<500 bp	935	47	43
Total	4118	561	546

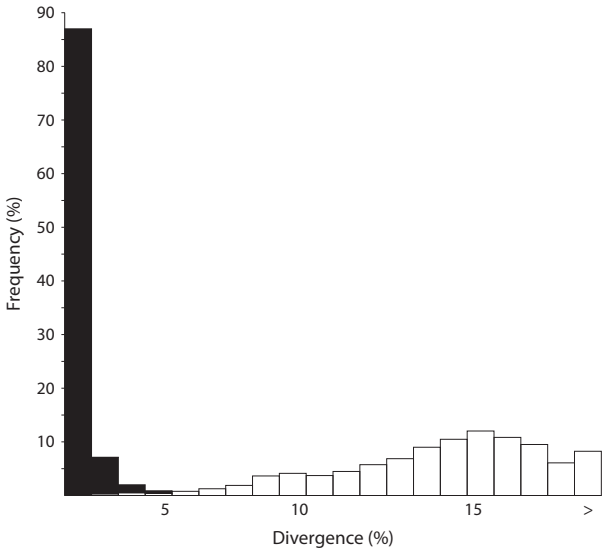

Fig. 1 Sequence divergence for all sequences compared at the species and genus levels. The histogram depicts the distribution of normalized divergence for species (black) against the genus divergences (white).

with unexpected BIN divergence in Central Europe, (2) species with unexpected BIN divergence between Central and Southern Europe and (3) species with BIN divergence that have been recognized as a possible complex. Species involved in BIN sharing are considered in two groups: (4) species that are known to be taxonomically problematic and (5) species that are generally accepted. Because some species or genetic clusters are represented by few specimens, some of the present conclusions are provisional. In particular, more specimens need to be analysed to assess the level of genetic variation and the status of genetic clusters across populations in Central Europe and elsewhere.

Species with BIN divergence in Central Europe—Specimens of 25 species from Central Europe were assigned to two or more BINs (Table 2). These cases of deep divergence were unexpected because no prior work has suggested the presence of sibling species in these taxa. More detailed morphological and genetic studies are required on each taxon to clarify its status. Appendix S4 (Supporting information) provides details on BIN assignments and divergences.

Species with BIN divergence between Central and Southern Europe—This study examined specimens of 191 species from both Central and Southern Europe. Specimens from 23 of these species were assigned to a different BIN in Southern than Central Europe (Table 3), indicating either the occurrence of substantial regional variation in barcode sequences or the presence of species overlooked by current taxonomy.

Taxonomically problematic sibling species with BIN divergence—Many species complexes of bees are controversial in Central Europe. For example, Stoeckhert (in Schmiedeknecht 1930) described a number of sibling species that were later synonymized, but several are now accepted as valid. However, the taxonomic status of other species remains uncertain (e.g. Amiet *et al*. 2010; Schmid-Egger 2012). This section considers how DNA barcodes help to clarify the taxonomy of 15 problematic species groups.

Andrena bicolor species group—The three species (*Andrena bicolor* Fabricius, 1775, *A. montana* Warncke, 1973, *A. allosa* Warncke, 1975) in this group are controversial (see Schmid-Egger 2012 for further details). The fact that each species shows clear barcode divergence and is assigned to a different BIN supports the hypothesis that each represents a good species. In fact, *A. bicolor* exhibits 4.0% intraspecific variation, suggesting that it may be a species pair.

Andrena bimaculata species group—The *Andrena bimaculata* species group is often thought (e.g. Schmid-Egger & Scheuchl 1997) to include four taxa as *Andrena bimaculata* species group [*A. bimaculata* (Kirby, 1802), *A. bluethgeni* E. Stoeckhert, 1930, *A. morawitzi* Thomson, 1872, *A. tibialis* (Kirby, 1802)]. *Andrena tibialis* and *A. morawitzi* are morphologically distinct, but the latter species only occurs in northern parts of Central Europe to Russia. The species status of *A. bluethgeni* is unclear (Schmid-Egger 2012); it is sometimes viewed as a colour form of *A. bimaculata*. Females of *A. tibialis* from Southern Europe are morphologically very similar to those of *A. bimaculata*. Barcode analysis revealed that species in this complex were assigned to three BINs. Males of *A. tibialis* from southern Germany (BOLD:ACE5720) possessed the large penis valve, which is diagnostic for that species. However, one female in this BIN from western Germany (Worms) possessed the dark facial setae and dark hind tibiae typical of *A. bimaculata*. The taxon that is usually regarded as *A. bimaculata* is probably represented by

Table 2 25 bee species from Central Europe that were assigned to two or more BINs. Percentage of divergence represents the maximum sequence distance (K2P) between specimens in the BINs comprising a species

No	Species	No. of BINs	% Divergence	Sympatric	Geographic origin	BIN
1	*Andrena bicolor**	2	3.98	Yes	France, Italy	BOLD:AAD0134
					Germany, France	BOLD:AAD0135
2	*Andrena congruens*	2	3.28	Yes	Italy	BOLD:AAF0994
					Italy	BOLD:ACG1524
3	*Andrena fulvida*	2	3.19	Yes	Germany	BOLD:AAK0294
					Germany	BOLD:ABX9995
4	*Andrena humilis*	2	3.76	Yes	Germany	BOLD:AAP2740
					Germany	BOLD:AAK0283
5	*Andrena lagopus*	2	10.18	No	France	BOLD:AAK0222
					Czech Republic	BOLD:ACC2245
6	*Andrena symphyti*†	2	2.53	No	Czech Republic	BOLD:ACH4274
					Germany	BOLD:ABX9246
7	*Andrena ventralis*	2	2.93	No	Germany (east)	BOLD:ABW9239
					Germany (south)	BOLD:ACC4196
8	*Anthidium loti*‡	2	2.95	No	Italy	BOLD:ABU8896
					France	BOLD:ACG1108
9	*Anthophora plagiata*§	2	2.18	No	Germany	BOLD:AAJ2561
					Italy	BOLD:ACJ8654
10	*Anthophora plumipes*	2	3.11	Yes	Germany	BOLD:AAF1672
					Germany	BOLD:AAF1671
11	*Dasypoda hirtipes*¶	2	5.25	Yes	Austria, Germany	BOLD:AAI9629
					Germany	BOLD:ABY4007
12	*Dufourea alpina***	3	11.40	No	Germany, Italy	BOLD:AAL1825
					Italy	BOLD:AAO3583
					France	BOLD:AAY5622
13	*Hoplitis leucomelana*	2	2.06	Yes	Germany, France, Italy	BOLD:AAK5812
					Germany, France	BOLD:ACF2274
14	*Hylaeus moricei*	2	3.77	No	Germany (south)	BOLD:AAE5084
					Germany (east), Czech Republic	BOLD:AAE5085
15	*Lasioglossum albipes*	3	3.31	Yes	Germany	BOLD:ABX6337
					Germany	BOLD:AAB0351
					Germany, Italy	BOLD:ACH2774
16	*Lasioglossum fulvicorne*††	5	5.46	(Yes)	Germany, France	BOLD:ACC1548
					Germany, France	BOLD:AAY5432
					Germany	BOLD:AAY9779
					Germany	BOLD:ACC1547
					Germany	BOLD:ACC1359
17	*Lasioglossum laticeps*‡‡	2	2.17	Yes	Germany, Italy	BOLD:AAY5433
					Germany	BOLD:ABZ5457
18	*Lasioglossum lativentre*	2	8.08	No	Germany (south)	BOLD:AAE1129
					Germany (east)	BOLD:ACH3798
19	*Lasioglossum villosulum*	3	8.83	Yes	Germany	BOLD:AAB7658
					Germany	BOLD:AAF3834
					Germany, France	BOLD:AAC2461
20	*Macropis fulvipes*	2	8.75	Yes	Germany	BOLD:AAJ1462
					Germany	BOLD:AAP1589
21	*Megachile willughbiella*	2	1.86	Yes	Germany, France	BOLD:ABZ3482
					Germany, Italy	BOLD:ACE6545
22	*Melecta albifrons*	3	6.48	No	Germany	BOLD:ABW1800
					Poland	BOLD:AAN9630
					Poland	BOLD:AAN9629
23	*Nomada lathburiana*	2	3.30	Yes	Germany	BOLD:AAF3659
					Germany	BOLD:AAF3660
24	*Nomada apaca*	2	6.36	Yes	Germany	BOLD:ABY3088
					Germany	BOLD:ACF5965

Table 2 (Continued)

No	Species	No. of BINs	% Divergence	Sympatric	Geographic origin	BIN
25	*Osmia mustelina*	2	2.54	Yes	Germany, Italy Germany	BOLD:AAE4127 BOLD:AAE4126

**Andrena bicolor* s.l. is a bivoltine species (Schmid-Egger & Scheuchl 1997) which may include a sibling taxon. Both lineages were represented in the spring and summer generations, ruling out the possibility that they are phenologically distinct taxa.
†A single specimen from Brandenburg in north-east Germany differed genetically (2.9%) from all other specimens which originated from southern Germany (including Thuringia).
‡The specimens in one BIN originated from Veneto province in north-eastern Italy, while those in the second BIN derived from south-western France.
§Representatives of the two BINs originate from northern Germany and northern Italy, sites about 1000 km apart.
¶Barcode results indicated three BINs with most German specimens belonging to the BIN BOLD:AAI9629 which represents the typical, most widespread type of this species. A single specimen from eastern Brandenburg and two specimens from Austria differ markedly (14.7%, BOLD:ABA8609, Appendix S1, Supporting information). As these specimens exhibit morphological differences from typical *D. hirtipes*, we conclude they represent a different species. The specimens probably belong to an eastern European species of *Dasypoda* (A. Dubitzky & C. Schmid-Egger, in preparation). The taxonomic status of two males from Mecklenburg-Vorpommern in northern Germany that were assigned to a third BIN (BOLD:ABY4007) is currently unclear.
**The NJ-tree indicates geographical subclustering (Alps in southern France/central part of western Alps/Southern Alps, cf. Appendix S7, Supporting information).
††The species included five BINs in the study area, but three were only represented by singletons. The NJ-tree suggested some geographic subclustering of BINs into northern and southern populations (cf. Appendix S7, Supporting information).
‡‡The species consists of two BINs, one from Rhineland-Palatinate and northern Italy and the other from several regions of Germany.

Table 3 23 bee species with BIN divergence in Southern Europe and between populations in different regions of Europe (S = Southern, N = Northern, C = Central)

Species	BIN 1	BIN 2	BIN 3	BIN 4
Andrena labiata Fabricius, 1781	Germany, Ukraine, N-Italy, S-France	S-France, N-Italy		
Colletes nasutus Smith, 1853	Germany	Turkey		
Halictus maculatus Smith, 1848	Germany, France, Italy	Italy		
Halictus smaragdulus Vachal, 1895	Germany, N-France	Cyprus	Greece	N-Italy
Hoplitis acuticornis (Dufour & Perris, 1840)	Germany, N-Italy	N-Italy, Turkey, Spain		
Hoplitis papaveris (Latreille, 1799)	Germany	S-France		
Hylaeus intermedius Förster, 1871*	S-France	Turkey		
Lasioglossum griseolum (Morawitz, 1872)	Germany, N-Italy, Croatia	S-France	C-Italy	
Lasioglossum interruptum (Panzer 1798)	Germany, Spain, S-France	Turkey		
Lasioglossum puncticolle (Morawitz, 1872)	Germany, Croatia	Turkey		
Lasioglossum pygmaeum (Schenck, 1853)	Germany	Croatia		
Lithurgus chrysurus Fonscolombe, 1834†	S-France	Hungary		
Megachile analis Nylander, 1852*	Sweden, Italy	Italy		
Nomada goodeniana/succincta	Germany	Croatia/Italy		
Nomada guttulata Schenck, 1861	Italy, France, Germany	Italy		
Nomioides minutissimus (Rossi, 1790)	Germany	Croatia		
Osmia andrenoides Spinola, 1808	Germany, S-France	Croatia, Italy, S-France		
Osmia aurulenta Panzer, 1799	Germany, S-France, Italy	Germany, Spain		
Osmia gallarum Spinola, 1808	Germany, S-France, Italy	Turkey		
Osmia versicolor Latreille, 1811*	S-France, N-Italy	Turkey	Turkey	S-Spain
Osmia viridana Morawitz, 1874*	N-Italy	Turkey		
Panurgus calcaratus (Scopoli, 1763)	Germany	N-Italy		
Pseudoanthidium scapulare Latreille, 1809	Germany, S-France	Turkey		

*No specimens from Germany available.
†Problematic taxon, see discussion below.

BOLD:AAY9952; it included one specimen from western (Hessen) and another from eastern Germany, identified as *A. bluethgeni*, as well as specimens from Turkey. The two male specimens possessed the small penis valve, and a face with dark setae typical of *A. bimaculata* and red hind tibia (a trait shared with *A. bluethgeni*). In addi-

tion, a third BIN (BOLD:AAK0349) included specimens from Bavaria, Baden-Württemberg and Thuringia. The male from Bavaria had a large penis valve, suggesting that it was *A. tibialis*, but the female in this BIN from Thuringia had a face with dark setae and dark hind tibiae, suggesting that it was *A. bimaculata*. The fourth species, *A. morawitzi*, was not available for study. The barcode results are confusing and reflect the taxonomic problems in this group. Perhaps the disagreement between traditional taxonomy and barcode results reflects hybridization, introgression or the presence of additional species.

Andrena proxima species group—Schmid-Egger (2005) recognized three species in this group: *Andrena ampla* Warncke, 1967, *A. alutacea* E. Stoeckhert, 1942 and *A. proxima* (Kirby 1802, see also Schmid-Egger 2012 for discussion). The barcode results support the validity of each of these species and revealed that several specimens from Saxony-Anhalt (central Germany) belonged to *A. ampla* rather than to *A. proxima* as initially thought. *A. ampla* is frequent in southern France and Spain, but these are the first records for Germany. The geographically closest populations of *A. ampla* occur in the Alps (Valais, Switzerland and Valle d'Aosta, Italy). Further range extensions can be expected as DNA barcoding gains broader use.

Andrena congruens species group—Schmid-Egger & Scheuchl (1997) and Schmid-Egger (2012) provide a detailed discussion of the species in this group, including *A. congruens* Schmiedeknecht, 1883 and *A. confinis* E. Stoeckhert, 1930). Despite their low (1.5%) interspecific divergence (Appendix S5, Supporting information), BOLD assigns these two species to separate BINs. In fact, *A. congruens* includes two BINs with substantial variation (3.3%, Appendix S4, Supporting information). As specimens of *A. confinis* were collected in Bavaria, while those of *A. congruens* originated from the Italian Alps, further specimens need to be analysed from sites where the species are sympatric to confirm their barcode divergence.

Andrena distinguenda species group—The *Andrena distinguenda* species group contains two BINs supporting the conclusion (Burger & Herrmann 2003) that it includes two species, *A. distinguenda* Schenck, 1853 s. s. and *A. nitidula* Pérez, 1903. The two species have differing distributions with *A. nitidula* in France, Spain and south-west Germany, whereas *A. distinguenda* is only known from Central Europe and Italy to South-Eastern Europe.

Andrena nitidiuscula species group—Schmid-Egger & Doczkal (1995) recognized two species in this group (*A. nitidiuscula* Schenck, 1853; *A. fulvicornis* Schenck, 1853) based on morphology, phenology and distribution. The barcode results confirm this conclusion with a distinct BIN for each taxon. Interestingly, specimens of *A. fulvicornis* from Germany and Turkey share the same BIN with very low sequence divergence (0.3%, Appendix S4, Supporting information) over this large distance.

Andrena ovatula species group—The *A. ovatula* group includes six species: *A. ovatula* (Kirby, 1802), *A. wilkella* (Kirby, 1802), *A. intermedia* Morawitz, 1870, *A. similis* Smith, 1849, *A. gelriae* van der Vecht, 1927 and *A. albofasciata* Thomson, 1870. Females in this group are very difficult to distinguish, but males are readily identified except those of *A. albofasciata* and *A. ovatula*, leading some taxonomists to regard them as conspecific (e.g. Amiet 2010). No specimens of *A. gelriae* were available for analysis, but barcodes assigned each of the other species to a different BIN except *A. albofasciata* that shared its BIN with *A. ovatula*, favouring their synonymy. All BIN assignments were based on the analysis of males, excepting *A. similis* where only a single female was available for analysis.

Andrena rhenana species group—Andrena rhenana E. Stoeckhert, 1930 and *A. taraxaci* Giraud, 1861 are well-established species with south-west Mediterranean and eastern Pontic distributions, respectively (Schmid-Egger & Scheuchl 1997). In Germany, *A. rhenana* only occurs in the upper Rhine Valley, whereas *A. taraxaci* is restricted to easternmost Bavaria (Schmid-Egger & Scheuchl 1997). Schwenninger (2007) described *A. pastellensis* as a sibling species of *A. taraxaci* from the Southern Alps. A specimen of *A. rhenana* from southern France was placed in a different BIN than the other two species, but *A. pastellensis* and *A. taraxaci* share a BIN. Four specimens from Bavaria and Czech Republic (all with short sequences) almost certainly represent the 'true' *A. taraxaci* because the type location of this species (Vienna, Austria) is in close proximity. Two specimens of this species from Greece, however, differ genetically from German specimens although the latter are only represented by short sequences. A single female from the Aosta Valley in north-western Italy (BC ZSM HYM 01400) clusters with *A. taraxaci* but shows slight sequence divergence from other specimens of this species and is identified as *A. rhenana* based on the description and distribution information by Schwenninger (2007), it probably represents *A. pastellensis*, albeit having the same BIN as *A. taraxaci*. Unfortunately, no males were available to substantiate the species status of *A. pastellensis.* Both morphology and the slight barcode divergence support recognition of *A. pastellensis* as a distinct species, although additional specimens from its eastern range

(i.e. Italy) and from other species in this complex require analysis to resolve the taxonomy of this group.

Halictus simplex Blüthgen, 1923, H. langobardicus Blüthgen, 1944 and H. eurygnathus Blüthgen, 1931—The *Halictus simplex* group includes three species in Central Europe. While the identification of females is difficult or impossible using morphology, males can readily be distinguished by the shape of their mandibles and genitalia. As a result, the following discussion only considers barcode data from males. Each of the three traditionally defined species included specimens that were placed in two or three BINs, and those of a particular species did not consistently cluster together (Table 4, Appendix S5, Supporting information). For example, specimens of *H. simplex* shared a BIN with two specimens of *H. eurygnathus* although these species seemed to show low sequence divergence (Appendix S6, Supporting information). Furthermore, a single male of *H. simplex* from Hungary differed clearly from German specimens of the same species, being assigned to a different BIN (BOLD: ACC2616). *Halictus langobardicus* included three BINs with two represented by singletons (a male from Germany and a female from southern France) (Appendix S6, Supporting information). Better taxon sampling is needed to clarify the status of this species complex as it may include more species than currently recognized.

Hylaeus gracilicornis species group—The validity of *Hylaeus gracilicornis* (Morawitz, 1867) and *H. paulus* Bridwell, 1919 has been controversial, but the two species are currently accepted as valid based on morphological and ecological differences (e.g. Amiet *et al*. 1999). The barcode data confirm their species status with a separate BIN for each taxon.

Hylaeus confusus species group—Straka & Bogusch (2011) revised this taxonomically difficult group and recognized three species: *Hylaeus gibbus* Saunders, 1850, *H. confusus* Nylander, 1852 and *H. incongruus* Förster, 1871. The barcode results confirm that each taxon is assigned to a distinct BIN. Interestingly, *H. gibbus* did not vary genetically, although specimens were examined from Hungary to north-western Italy, a distance of nearly 1000 km.

Lasioglossum fratellum species group—The two sibling species *Lasioglossum fratellum* (Pérez, 1903) and *L. subfulvicorne* (Blüthgen, 1934) are difficult to distinguish morphologically, particularly the females. However, they are accepted as valid species (Ebmer 1988) and the barcode results support this conclusion with each species in a separate BIN (Appendix S4, Supporting information).

Lasioglossum sexstrigatum species group—The group contains three species: *Lasioglossum sexstrigatum* (Schenck, 1870), *L. sabulosum* (Warncke, 1986) and *L. pleurospeculum* Herrmann 2001. Herrmann & Doczkal (1999) provided diagnostic morphological characters for *L. sexstrigatum* and *L. sabulosum*, while Herrmann (2001) described *L. pleurospeculum* from Central Europe. The barcode data confirm that *L. sexstrigatum* and *L. pleurospeculum* are distinct because they were assigned to different BINs. Although they lacked full-length sequences, four specimens of *L. sabulosum* with short sequences clustered separately from *L. pleurospeculum*, being very divergent from *L. sexstrigatum* (Appendix S5, Supporting information).

Lasioglossum nitidulum species group—Lasioglossum nitidulum (Fabricius, 1804) and *L. smeathmanellum* (Kirby, 1802) are difficult to distinguish by morphology, but are accepted as valid species (Ebmer 1988). *Lasioglossum nitidulum* was represented by nine full-length barcode sequences from specimens collected in Bavaria and northern Italy, while the single specimen of *L. smeathmanellum* from Portugal did not yield a full barcode sequence. However, the latter species showed considerable sequence divergence from *L. nitudulum*, appearing to be closer to *L. alpigenum* (Dalla Torre, 1877).

Nomada fulvicornis species group—Although the species status of *Nomada meridionalis* Schmiedeknecht, 1882 has

Table 4 Overview of species and associated BINs in the *Halictus simplex* species complex

BIN	Species (males only)	Origin	Number of specimens (including sequences < 500 bp)
BOLD:AAD 5869	*H. eurygnathus*	S-Germany	2 males, 1 female
BOLD:AAD 7942	*H. eurygnathus*	S-Germany, SE France	2 males, 1 female
BOLD:ACF 0661	*H. langobardicus*	SE-France	1 male
BOLD:ACE 8207	*H. langobardicus*	SW-Germany	21 males and females
BOLD:ACC 2616	*H. simplex*	Hungary	1 male
BOLD:AAD 5869	*H. simplex*	Germany, NW-Italy	16 males and females
BOLD:ABZ 9465	*Halictus* sp.	SW-Germany	1 female

been questioned (M. Schwarz, personal communication 2010), Doczkal & Schmid-Egger (1992) accepted it as a valid species because it differs morphologically from *N. fulvicornis* Fabricius, 1793, and these two species have divergent host preferences and phenology. Recent studies (D. Doczkal, personal communication 2010, Straka, personal communication 2014 and personal observations) indicate that *N. fulvicornis* is a complex of several species in Central Europe, each with a different host association. Barcode analysis revealed two BINs for *N. fulvicornis* in Germany, and a separate BIN for *N. meridionalis*, but only a single full-length sequence for the latter species was available. Based on examination of the short sequences, it appears that there is another BIN in southern Germany, two in eastern Germany (Berlin and Brandenburg) and another in the Czech Republic.

Nomada sheppardana species group—Nomada sheppardana (Kirby, 1802) and *N. minuscula* Nosciewicz, 1930 are difficult to distinguish by morphology, but are assigned to different BINs (Appendix S5, Supporting information), supporting their status as distinct species.

Taxonomically problematic species which share a BIN. The following section considers seven species of bees with problematic taxonomy. All of the species involved are morphologically very similar, and their taxonomic status has been controversial. In some species groups, the NJ tree shows subclusters, which suggests the validity of certain species, although there is <2% sequence divergence, but some of these cases may reflect geographic variation. Larger sample sizes are needed to gain a full understanding of the contribution that DNA barcoding can make to the resolution of these groups.

Andrena carantonica species group—The *A. carantonica* species group includes three taxa: *A. carantonica* Pérez, 1902, *A. spinigera* (Kirby, 1802) and *A. trimmerana* (Kirby, 1802). *Andrena trimmerana* and *A. spinigera* are not generally accepted as being different from *A. carantonica* (see Schmid-Egger 2012 for details). However, there may be two valid species in Central Europe, one univoltine and widespread (*A. carantonica*), and the other bivoltine and rare (*A. trimmerana*). Specimens assigned to *A. spinigera* may simply represent the first generation of *A. trimmerana*, a species that only occurs in the upper Rhine valley, a particularly warm region within Germany. Whereas females of *A. trimmerana* and *A. carantonica* cannot be separated by morphology, males can be distinguished by the shape of their mandibles (*A. trimmerana* has an subapical tooth while *A. carantonica* lacks it; Schmid-Egger & Scheuchl 1997). The three species in this complex share a BIN, but the specimens form two subclusters. One is composed of *A. trimmerana/spinigera* from Germany and the Czech Republic, while all specimens of *A. carantonica* form a second cluster (Appendix S6, Supporting information). The first cluster contains three specimens, a single female of *A. trimmerana* collected from Germany in summer, and two males from the Czech Republic, both collected in spring and identified as *A. spinigera*. These results suggest that *A. spinigera* and *A. trimmerana* are conspecific, representing the spring and summer generations of a species that is distinct from *A. carantonica*.

Andrena dorsata species group—The two accepted taxa in this species group, *A. dorsata* (Kirby, 1802) and *A. propinqua* Schenck, 1853 share the same BIN. The taxonomic status of the species in this group is controversial (Gusenleitner & Schwarz 2002; Amiet *et al.* 2010), and the separation of specimens through morphological criteria is sometimes uncertain. The barcode results reveal two subclusters with 1% divergence that are strongly associated with specimen identifications. If the few exceptions represent misidentifications, the barcode results would suggest the validity of these two species.

Andrena pilipes species group—The *A. pilipes* group includes two very similar species, *A. pilipes* Fabricius, 1781 and *A. nigrospina* Thomson, 1872. The species are distinguished by minor differences in male genitalia and the colour of the pubescens in females. Among 18 specimens barcoded from various parts of Germany, only a single male was reliably identified as *A. nigrospina*. Although it possessed an interspecific distance of 1.5% from the specimens of *A. pilipes,* both species were assigned to the same BIN. Although it is likely that these two species can be separated by barcodes, more specimens of *A. nigrospina* need to be analysed to confirm this conclusion.

Andrena rosae species group—The status of *Andrena rosae* Panzer, 1801 and *A. stragulata* Illiger, 1806 is controversial. A recent genetic study suggests that *A. stragulata* is the first generation of *A. rosae* (Reemer *et al.* 2008; see also Schmid-Egger 2012 for further details). Barcode data support this conclusion as no sequence divergence was detected, although *A. stragulata* was only represented by two short (287 bp) sequences from German specimens.

Epeolus cruciger species group—Epeolus marginatus Bischoff, 1930 is not generally recognized as a different species from *E. cruciger* Panzer, 1799 due to its lack of diagnostic morphological characters (F. Burger, personal communication 2011). However, host relationships (*E. cruciger* is a parasite of *Colletes succcintus* (Linnaeus, 1758) while *E. marginatus* parasitizes *Colletes marginatus* Smith, 1846) and phenology (*E. cruciger* occurs in August/September while *E. marginatus* flies in June/

July) are different, supporting recognition of the two species. However, their barcodes show no sequence divergence, suggesting that they may be a single taxon (Appendix S5, Supporting information).

Panurginus montanus species group—Panurginus herzi Morawitz, 1892, *P. montanus* Giraud, 1861 and *P. sericatus* (Warncke 1972) were long regarded as subspecies of *P. montanus* (Warncke 1972), but Amiet *et al.* (2010) recognized them as valid species. Because morphological discrimination of *P. montanus* and *P. sericatus* is difficult, the species are mainly separated based on their distribution. *Panurginus sericatus* occurs from France to Switzerland, *P. montanus* from eastern Switzerland eastwards, and *P. herzi* in the central and northern Alps. Specimens of *P. sericatus* from France, Italy and Switzerland, and *P. montanus* from the German Alps share the same BIN, and the single short sequence for *P. herzi* also showed close congruence with those of its two congeners, suggesting these are very young species (Appendix S7, Supporting information).

Stelis minuta species group—The validity of *Stelis minima* Schenck, 1861 and *S. minuta* Lepeletier and Serville, 1825 is not generally accepted (M. Schwarz, personal communication 2010). Because both species were represented by a single short sequence, full-length sequences are needed to confirm their lack of sequence divergence.

Taxonomically accepted but closely related species which share a BIN. Our analysis revealed 12 pairs and three trios of species which shared a BIN although they can be differentiated morphologically and often also by distribution pattern, phenology or ecology. Closer inspection of these cases revealed that just five species pairs shared sequences (marked with a * below). The others involved cases of low sequence divergence.

Andrena apicata Smith, 1847, A. batava Pérez, 1902 and A. mitis Schmiedeknecht, 1883—Andrena mitis, A. apicata and A. batava are taxonomically well established and can readily be distinguished by morphological characters. *Andrena mitis* was represented by 17 full-length barcode sequences and showed little variation (0.16%, Appendix S5, Supporting information). The short sequences for *A. apicata* and *A. batava* from Central Europe indicate that their barcodes are very close to those for *A. mitis*, but full-length barcodes for *A. apicata* from other parts of Europe indicate that it can be distinguished from *A. mitis*.

Andrena barbareae Panzer, 1805 and A. cineraria* (Linnaeus, 1758)—Andrena barbareae* occurs in the Southern Alps and is a sibling species of the widespread *A. cineraria*. The species have phenological differences as *A. cineraria* has a single generation per year, while *A. barbarea* is bivoltine. The species status of the latter has generally been accepted (e.g. Schmid-Egger & Scheuchl 1997), but the two species appear to share DNA barcodes.

Andrena limata Smith, 1853, A. nitida* (Müller, 1776) and A. thoracica (Fabricius, 1775)—Andrena nitida* is a widespread, common species in Germany, while *A. thoracica* is known from a single location near Berlin, and *A. limata* is restricted to the extreme south-west of Germany. These species are taxonomically well accepted and can be distinguished primarily by their setal coloration. The three species are assigned to the same BIN (Appendix S7, Supporting information) with specimens of *A. limata* and *A. nitida* sharing identical sequences. Specimens of *A. thoracica* from Central Europe show about 0.9% divergence from the other two species so they can be recognized by barcodes (Appendix S5, Supporting information). Two lineages of *A. thoracia* were detected in Turkey, one identical to that in Germany and the other showing 3.1% divergence, suggesting the presence of cryptic species.

Colletes hederae Schmidt and Westrich, 1993 and C. succinctus* (Linnaeus, 1758)—Colletes hederae* and *C. succinctus* are well-established species (Kuhlmann *et al.* 2007), but a single full-length barcode sequences for *C. succinctus* was identical to two sequences for *C. hederae*. Kuhlmann (personal communication 2010) described the *C. succinctus* complex as 'very young', which may explain their lack of barcode divergence.

Epeolus alpinus Bischoff, 1930 and E. schummeli Schilling, 1849—Epeolus schummeli, a parasite of *Colletes nasutus* Smith, 1853, occurs in Eastern Europe including a small area in eastern Brandenburg in Germany. By contrast, *E. alpinus*, is a boreo-alpine species, found in the Alps and in northern Europe where it is parasitic on *Colletes impunctatus* and *C. floralis* Eversmann, 1852. Barcodes from a single specimen of *E. schummeli* and two specimens of *E. alpinus* were assigned to the same BIN, but there was evidence of diagnostic sequence differences with a genetic distance of 0.49% between the taxa (Appendix S7, Supporting information).

Hylaeus alpinus (Morawitz, 1867) and H. hyalinatus Smith, 1842—Hylaeus alpinus is an alpine species, whereas *H. hyalinatus* is common and widely distributed in Europe except in montane settings. The two species are difficult to distinguish morphologically, and they share a BIN. However, they do not appear to share barcode sequences, as they possessed an average interspecific divergence of 0.74% (Appendix S5, Supporting information).

Hylaeus brevicornis Nylander, 1852 and H. glacialis Morawitz, 1872—*Hylaeus glacialis* is restricted to the Alps, while *H. brevicornis* is a common and widespread xerothermic species in Central Europe. The single barcoded male of *H. glacialis* from France was placed in the same BIN as nine specimens of *H. brevicornis* from Germany and Hungary. However, it showed more than 1.1% sequence divergence from the nearest specimen of *H. brevicornis*, indicating that the two species can be distinguished through barcodes. *Hylaeus gredleri* Förster, 1871, is another morphologically similar species, but it shows marked barcode divergence from *H. brevicornis* and *H. glacialis* and is placed in a different BIN.

Hylaeus pfankuchi (Alfken, 1919) and H. rinki (Gorski, 1852)—Although morphologically very similar, the males of these two species are not difficult to distinguish (Amiet *et al*. 1999). The barcode results place the two species in the same BIN, but they show clear sequence divergence (1.3%, Appendix S7, Supporting information).

Lasioglossum bavaricum (Blüthgen, 1930) and L. cupromicans* (Pérez, 1903)*—These two species are difficult to separate and only females were barcoded, but they were identified by the leading specialist for this group (A. W. Ebmer, Puchenau, Austria). *Lasioglossum bavaricum* was only represented by two specimens with short sequences (421 bp), but they were identical with sequences from *L. cupromicans*, suggesting that these species lack barcode divergence.

Nomada flava Panzer 1798, N. ferruginata (Linnaeus, 1767), N. glabella Thomson, 1870 and N. leucophthalma (Kirby, 1802)—All species in this complex (except *N. glabella*) are generally accepted by taxonomists. Aside from these four species, *N. panzeri* Lepeletier, 1841 shows close morphological similarity to *N. flava*. *Nomada glabella* is taxonomically problematic and is usually viewed as a synonym of either *N. flava* or *N. panzeri*. The barcode results reveal that *N. panzeri* is genetically very distinct as it is assigned to a different BIN (Appendix S5, Supporting information). Although the other three species in this complex (except *N. glabella*) are straightforward to identify, they share the same BIN (Appendix S7, Supporting information). However, specimens of *N. ferruginata*, *N. flava*, *N. glabella* and *N. leucophthalma* each form a distinct subcluster within the BIN (excepting a single specimen of *N. glabella* that clusters with *N. leucophthalma*, a situation that requires further investigation).

Nomada goodeniana (Kirby, 1802), N. succincta* Panzer 1798*—Although these species are generally accepted as valid (e.g. Kuhlmann 1997), their separation is difficult as it is largely based on (variable) colour differences. Specimens of both species from Central Europe share the same BIN (Appendix S5, Supporting information), with no discernible subclustering. However, specimens of *N. succincta* from Southern Europe (two specimens from Croatia and the Aosta Valley in north-western Italy) show clear barcode divergence from Central European populations coupled with intraspecific variation of 2.8% (Appendix S5, Supporting information), suggesting the presence of cryptic species. Several specimens identified as *N. succincta* from Finland, Italy (Aosta Valley) and Croatia form one cluster (BOLD:ABX5010), whereas a second cluster (BOLD:AAE1973) consisted of specimens identified as *N. succincta* or *N. goodeniana* from Central Europe. *Nomada succincta* was originally described from Austria (Panzer 1798), suggesting that the German specimens represent *N. succincta sensu* Panzer while the Finnish and Southern European populations belong to separate species.

Nomada roberjeotiana Panzer, 1799 and Nomada tormentillae Alfken, 1901—The distinctiveness of *Nomada roberjeotiana* Panzer, 1799 and *Nomada tormentillae* Alfken, 1901 has generally been accepted (Tkalcu 1974; Scheuchl 1995), but Amiet *et al.* (2007) treat both species as conspecific. The two species differ in morphology, distribution and host relationships. *Nomada roberjeotiana* is a lowland species, occurring in sandy habitats and associated with *Andrena denticulata* (Kirby, 1802) and *A. fuscipes* (Kirby, 1802) (Scheuchl 1995), while *N. tormentillae* is a montane species that parasitizes *Andrena tarsata* Nylander, 1848 (Scheuchl 1995). The two species share a BIN (Appendix S7, Supporting information), but possess diagnostic sequence differences with a minimum divergence of 0.7%.

Sphecodes alternatus Smith, 1853 and S. crassanus Warncke, 1992—These two species are morphologically difficult to separate, but there is little doubt about their status as distinct species (Bogusch & Straka 2012). Although they share a BIN (Appendix S7, Supporting information), the taxa show 1.1% sequence divergence suggesting that they can be diagnosed with barcodes, but more sequences are required to confirm this conclusion.

Sphecodes crassus Thomson, 1870 species complex—The *S. crassus* group includes seven species that are difficult to discriminate morphologically, but are generally accepted as valid (Bogusch & Straka 2012). No specimens of *S. pseudofasciatus* Blüthgen, 1925 or *S. zangherii* Noskiewicz, 1931 were analysed, but the other five species possessed distinct barcode sequences. Three (*S. geofrellus* Kirby 1802; *S. marginatus* von Hagens, 1882; *S. miniatus* von Hagens, 1882) were assigned to different BINs (Appendix S5, Supporting information), supporting their

status as good species. The final pair of taxa, *S. crassus* and *S. croaticus*, shared a BIN but formed distinct subclusters with a minimum sequence divergence of 1.48% suggesting that they also represent valid taxa (Appendix S5, Supporting information).

Sphecodes ferruginatus von Hagens, 1882 and S. hyalinatus von Hagens, 1882—Although their morphological differences are small, *S. ferruginatus* and *S. hyalinatus* are accepted as good species (Bogusch & Straka 2012). These species share a BIN, but the specimens of each species form a distinct subcluster with a minimum sequence divergence of 1.08% so they can be discriminated by barcodes. (Appendices S7 and S6, Supporting information).

Discussion

This study begins the assembly of a DNA barcode library for European bees, providing records for 4118 specimens, representing 561 species (Table 5). This total includes records for 546 of the 571 species (96%) recorded from Germany, although coverage for 43 German species is based on sequences that include only a portion of the barcode region (Table 1, Appendix S3, Supporting information). Several species from adjacent areas were also analysed because of their possible occurrence or future potential for range expansion into Germany. In fact, this study revealed the presence of *Andrena ampla* in Germany, a species previously known only from southern France and Spain. Most of the species lacking barcodes are rare taxa represented only by old museum specimens, but recently collected specimens of some species also failed. Inspection of trace files indicated that most of these failures arose from co-amplification of the bacterial endosymbiont *Wolbachia*, which impeded interpretation of the trace files. Primer matching was likely poor for these species, a problem which can likely be overcome by their redesign. In fact, the bee family Halictidae appears to have a relatively high prevalence of *Wolbachia*, possibly because one of the standard insect primers (LepR1) has a better fit with the bacterial endosymbiont than with the insect host (Smith *et al.* 2012).

Table 5 DNA barcoding success for bees of Germany and Europe (except Germany). For details see Appendices S4, S5 and S7 (Supporting information)

Total	Number of sequences recovered	4118
	Number of species with sequences	561
	Number of species with sequences > 500 bp	514
	Number of species with sequences < 500 bp	47
	Number of BINs	557
	Number of species with barcode sharing	50
	Number of species with BIN divergence	56
Germany	Number of species recorded from Germany	571
	Number of German species with sequences > 500 bp	503
Extralimital	Number of species with sequences > 500 bp	11

It has been estimated that *Wolbachia* is present in two-third of all insect species (Hilgenboecker *et al.* 2008) and between one-fifth and three-quarters of all bees include at least some *Wolbachia*-infected individuals (Gerth *et al.* 2011; Stahlhut *et al.* 2012). Infections with *Wolbachia* can lead to underestimating diversity (fixation of one species' mtDNA in closely related species through mitochondrial introgression; Whitworth *et al.* 2007; Raychoudhury *et al.* 2009) or overestimating diversity (infection of a species with different *Wolbachia* strains or partial infection of a species; Whitworth *et al.* 2007; Xiao *et al.* 2012). Despite the common occurrence of *Wolbachia* in insects and other arthropods, the present study and other taxon-specific studies have demonstrated that it has no or little effect on the delimitation of species through DNA barcodes (Linares *et al.* 2009; Smith *et al.* 2007, 2012; Stahlhut *et al.* 2012).

Among the 514 species with full-length barcode sequences, 56 (10.9%, Table 5) showed deep genetic divergence. As a result, their component specimens were assigned to two (45 species), three (eight species), four (two species) or five (one species) BINs (Appendix S4, Supporting information). By contrast, 50 species (9.7%, Table 5) possessed such low interspecific divergence that they shared a BIN with one or more species (Appendix S7, Supporting information). However, most of the species involved in BIN sharing possessed sequence differences that allowed the discrimination of the individuals belonging to the different species within a particular BIN. In all cases, the barcode results enabled the assignment of 'unknown' specimens to a particular species complex.

Five species that shared their BIN with another species also included specimens that were assigned to one or more additional BINs (Appendix S7, Supporting information). All of these cases involved taxonomically problematic species groups (e.g. *Andrena bimaculata*, *A. tibialis*, *Nomada succincta*, *Halictus eurygnathus*, *H. simplex*) and perhaps reflect cases where additional cryptic species are present. However, these cases need to be examined for the effects of *Wolbachia* infections. The overall results indicated that the 514 species of bees identified using morphological characters included representatives of 557 BINs. The detection of 43 more BINs than the number of traditionally accepted species suggests that DNA barcoding will increase taxonomic resolution

by overlooked species complexes. The results indicate a higher number of bee species in Germany compared to current checklists, but the cases of cryptic diversity need to be examined in detail before conclusions can be drawn about the implications for the current estimate of bee species in Germany. Putative cases of cryptic diversity, in particular in taxonomically problematic species complexes, as observed in several species of *Andrena*, *Nomada*, *Halictus*, *Lasioglossum* and *Osmia*, can benefit from a supplementary nuclear marker in addition to the barcode fragment (Neumeyer *et al.* 2014).

The present study helped to clarify the taxonomic status of more than 30 species of bees, representing 15 groups of sibling species, many of which have been controversial among taxonomists for at least a half century. Difficult species groups benefitted particularly because identifications by DNA barcodes were often more reliable than those based on morphology. In fact, nearly 1% of the specimens examined in this study were found to have been misidentified, although all had been examined by specialists. Some misidentifications involving species that are easily discriminated were caused by 'clerical' mistakes, but other errors reflected uncertainty in the amount of intraspecific variation in diagnostic characters in difficult species groups (e.g. *Lasioglossum lucidulum* group, some *Sphecodes* species, males of the *Coelioxys mandibularis* group). In these cases, the barcode results aided re-evaluation of the morphological characters employed for species-level identification.

Traditional taxonomy in the light of DNA barcoding

The traditional taxonomy of bees has, as in most other groups, been primarily based on the examination of morphological characters, occasionally supplemented by information on phenology, plant associations and other ecological information and geographic distribution (Winston 1999; Packer *et al.* 2009). However, the detection of morphological discontinuities has usually been the first step in recognizing species. The assessment of taxonomically informative characters and the detection of sometimes subtle morphological differences among species requires years of experience. Even then, the study of morphology includes a subjective component, as exemplified by longstanding uncertainty regarding the status of certain taxa, such as the cases discussed earlier in this publication. Because many morphological characters are difficult to describe, illustrations (e.g. line drawings, photographs) are often critical for the evaluation of diagnostic differences between species. However, even when high-quality illustrations are available, the subtle morphological differences which separate some species are often difficult to assess unambiguously, even microscopically, because of variation in optics, lighting, specimen wear and experience of the researcher (Packer *et al.* 2009). Well-illustrated identification keys are a minimum requirement for reliable identifications but, even if they were available for all bee taxa, they would not allow the identification of all bees to species level because this often requires access to a reference collection with reliably identified specimens for comparison.

DNA barcoding and previous species concepts in bees

Because the barcode reference library generated in this study provides coverage for most Central European bee species, it provides an opportunity to examine the correspondence between the taxonomy of bees, a tradition begun in the 18th century, and the results from DNA barcoding. This comparison shows that taxa recognized through traditional taxonomic studies correspond well with COI sequence clusters as delineated by BINs. In fact, our results establish that nearly 90% of the species of bees known from Germany were assigned to a distinct BIN because of their deep sequence divergence from any other taxon. Within a species, DNA barcodes usually varied by less than 1%, even when the comparison involved populations from localities as distant as Berlin, Bavaria and the Southern Alps. This pattern appears to extend to a European scale, although the inclusion of island populations (e.g. Sicily, Sardinia) reveals slightly higher intraspecific variation. Our results also indicated that DNA barcoding often helps to resolve species in groups that are difficult to separate morphologically or that have other taxonomic problems. Our results confirm the effectiveness of DNA barcodes in the discrimination of bee species, reinforcing conclusions from work on bees in other regions and on other groups of insects in Germany. For example, studies on the bees of Ireland (Magnacca & Brown 2012) and of Nova Scotia (Sheffield *et al.* 2009) showed that DNA barcodes were highly effective in species identification. A recent study demonstrates how barcodes can help support morphology-based taxonomy in one of the taxonomically most challenging groups of bees (Gibbs *et al.* 2013, see also http://bee-bol.org/). In a similar way, work on geometrid moths revealed that 93% of species possessed diagnostic barcode sequences at sites across Europe and that 97% were distinct in sympatry (Hausmann *et al.* 2013a,b). Where barcodes fail to deliver species resolution or where they reveal taxonomic conflicts, detailed evaluations of additional specimens and additional gene regions will be necessary.

Resolution of sister taxa

The results from the barcode analysis of pairs or groups of species whose status is contested was varied. Some species problematic to separate through traditional

approaches were well differentiated by DNA barcodes. In such cases, the new data should often resolve the status of presumptive taxa in a decisive fashion. However, other presumptive species pairs, such as *Epeolus cruciger* and *E. marginatus,* lacked divergence in the barcode region. Cases such as these require further study, involving the analysis of more specimens and additional populations. When such studies do not provide evidence of barcode divergence, this should not be viewed as proof that the taxa are a single species (e.g. Burns *et al.* 2007). In cases where other information supports the presence of two or more taxa, additional genes should be analysed to examine their differentiation (e.g. Dupuis *et al.* 2012). In addition, examination of sequencing trace files not only aids in verifying barcode sequences and confirming sequence integrity, but their inspection can reveal sequence inconsistencies and irregularities when barcoding yielded different results than traditional taxonomy.

Discovery of new sister species

Our results indicate that Central and Southern European populations of a particular species often show marked sequence divergence at COI, suggesting that many pairs of sister species exist where current taxonomy assumes a single taxon. Among just over 500 species of bees, we found 23 with COI divergences exceeding 2%, a level of sequence divergence that often signals different species (see Table 3). Further examination using integrative taxonomic approaches (e.g. Rafter *et al.* 2013) is required to ascertain if these cases reflect cryptic species. It needs emphasis that the use of a sequence threshold to target species deserving of detailed study is best applied in cases of sympatric divergence, not to populations that are distantly allopatric [see Mutanen *et al.* (2012) for a discussion of genetic divergence, allopatry and species status in Arctic–Alpine Lepidoptera].

The role of DNA barcoding in current taxonomy

A DNA barcode reference library is only as good as the taxon sampling that underpins it and the care with which the specimens have been identified. If a particular group lacks taxonomic specialists, or if the identifications of barcoded individuals have not been properly validated, BOLD cannot deliver reliable species identifications. Because the work involved in constructing a comprehensive barcode library for all animal species is large, species of economic importance form a logical first target. The capacity to apply DNA barcoding for the identification of specimens is already well developed in some groups (e.g. in Lepidoptera, for which taxon sampling has reached about 50% of all known species). If a researcher wishes to activate work on a diverse insect assemblage in a little studied geographic region, DNA barcoding is invaluable (e.g. Janzen *et al.* 2009). Using this approach, specimens are barcoded first and subsequently analysed morphologically. Barcode clusters facilitate detailed taxonomic study by indicating which individuals are likely to belong to a single species and by associating males and females in dimorphic taxa.

DNA barcoding will not render taxonomists obsolete as it does not aim to reach final conclusions on species status. Taxonomic researchers retain responsibility for reaching a decision based upon the total body of evidence. As a result, taxonomists will continue to generate hypotheses on species limits and to decide which genetic entities constitute a species and which do not. Measures of sequence divergence will increasingly aid these decisions, but they will not relieve the taxonomist of interpreting the data and reaching a decision. However, DNA barcoding will make this task significantly easier to accomplish as revealed by the fact that few taxonomic studies proceed without genetic data, especially in difficult groups of organisms. Molecular analyses will become increasingly important as taxonomists consider groups of organisms that have been neglected, even in well-studied areas like Central Europe. Among insects, these groups include most taxa with small body size, high species richness and consequently lacking keys for their identification. Examples include the Diptera and Hymenoptera that together comprise, with nearly 20 000 recorded species, over 50% of the German insect fauna. The extent of taxonomic uncertainty in such groups is often unappreciated. For example, published host-parasitoid records are highly unreliable because in 75% of these cases either the parasitoid or the host was misidentified (Noyes 1994).

The changing image of taxonomy

In Europe, insect taxonomy has traditionally been advanced largely by amateurs (Fontaine *et al.* 2012) who have described most known species. This approach has now led to the situation where taxonomy is recruiting few new researchers, leading to a decline in taxonomic capacity (e.g. House of Lords 2008). At the same time, new imaging and analytical methods are providing remarkable opportunities for progress. Recent technological advances in digital imaging technology of individual specimens (Nguyen *et al.* 2014) and whole insect drawers (Schmidt *et al.* 2012) will, for the first time, provide access for both expert scientists and the general public to the world's natural history collections (Blagoderov *et al.* 2012; Balke *et al.* 2013). Sophisticated software for data analysis and phylogenetic reconstruction, electronic publishing, and molecular genetic techniques are now standard tools for entomological taxonomy. Naturally, these changes have alienated some taxonomists employing traditional

approaches. Every new force has to overcome inertia, and revolutions meet with resistance in entomology as elsewhere in life. Clearly, some criticisms of DNA barcoding are rooted in resistance to change. Although it is only one of several new approaches being deployed in entomology, it is the first molecular genetic approach that has been applied to a large number of species. This, and its utility, as illustrated by the results presented here mean that it provides an opportunity for progress. In fact, novel citizen science initiatives such as LifeScanner will allow the public to become engaged in the discovery and identification of organisms using DNA barcoding technology (www.scientificamerican.com/citizen-science/lifescanner).

As opposed to a threat, we see DNA barcoding as a great opportunity for both amateur and professional entomologists to contribute to the progress of science by adding records to the Web-accessible BOLD, arguably the most important tool that is globally available for taxonomic research. Based on our experience, insect taxonomy has been held in rather low esteem for decades, a major reason for systematics losing academic participation and resources. External perceptions of taxonomy and its capacity to deliver new scientific insights will be advanced through the large-scale adoption of new technologies, particularly genetic approaches, provoking the funding and academic interest which will reinvigorate taxonomy as a major field in entomology.

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S.S., C.S.-E. and J.M. conceived the study and conducted the molecular and taxonomic analyses. S.S., C.S.-E., J.M., G.H. and P.D.N.H. wrote the manuscript.

Data accessibility

All specimen data are accessible on BOLD (www.boldsystems.org) through the following DOIs: dx.doi.org/10.5883/DS-GBAPI and dx.doi.org/10.5883/DS-GBAPS. The data include collection locality, geographic coordinates, altitude, collector, one or more images, identifier and voucher depository. Sequence data are available on BOLD including a detailed LIMS report, primer information and trace files and also on GenBank.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. List of voucher specimens with sample ID, barcode index number (if present), country of origin, and GenBank accession number.

Appendix S2. German species not available for barcode analysis.

Appendix S3. 43 German species represented by non-barcode compliant sequences.

Appendix S4. 56 species of bees that exhibit BIN divergence (for acronyms see Appendix S5, Supporting information).

Appendix S5. Barcoding statistics of bee species with distribution, barcode index number (BIN), number of specimens, mean intraspecific distance, maximum intraspecific distance, barcoding statistics, nearest neighbour species and distance to nearest species.

Appendix S6. BOLD TaxonID Tree.

Appendix S7. 49 species of bees that exhibit BIN sharing (for acronyms see Appendix S5, Supporting information).