

Recognition and identification of bumblebee species in the *Bombus lucorum*-complex (Hymenoptera, Apidae) – A review and outlook

Silas Bossert¹

¹ Department of Integrative Zoology, University of Vienna, Althanstraße 14, UZA 1, 1090 Vienna, Austria.

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Corresponding author: Silas Bossert (silas.bossert@googlemail.com)

Abstract

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The recognition of cryptic species represents one of the major challenges in current taxonomy and affects our understanding of global diversity. In practice, the process from discovery to acceptance in the scientific community can take an extensive length of time. A prime example is the traditionally difficult taxonomy of the cryptic bumblebee species belonging to the *Bombus lucorum*-complex. The status of the three European species in the group – *Bombus lucorum* and the closely related *Bombus cryptarum* and *Bombus magnus* – has recently become widely accepted, primarily due to investigations of nucleotide sequences and marking pheromones. In contrast, doubts prevail concerning the validity of species identification based on morphology. As a consequence, our knowledge of the species is muddled in a mire of unreliable and confusing literature data from a large number of authors over the centuries. To clarify this issue, this paper provides a recapitulation of the historical literature and highlights the milestones in the process of species recognition. Further, the possibility of a morphologically based species identification is discussed in the context of new molecular data. Finally, this review outlines the current challenges and provides directions for future issues.

Introduction

Bumblebees (*Bombus* Latreille, 1802) are considered to be a striking feature of Europe's pollinator fauna (e.g., Corbet et al. 1991, Neumayer and Paulus 1999, Goulson et al. 2007, Goulson 2010). In contrast to most other bee genera, bumblebees are readily recognizable and rarely confused with other bees (Amiet 1996, Gokcezaade et al. 2010, Amiet and Krebs 2012). Yet species determination requires expertise, and reliable identification in the field is often impossible. Reasons for this are the relatively monotonous morphology (Michener 2007), enormous variability in coloration and size which is often associated with biogeographical distribution (e.g., Vogt 1909, Vogt 1911, Krüger 1951, Løken 1973, Pekkarinen 1979) and the fact that the same or similar color-patterns are often repeated in various species (Dalla Torre 1880, Reinig 1939, Amiet 1996, Williams 2007 and references therein). One of these groups with very similar morphology consists of the European species of the subgenus *Bombus*

s. str.: *Bombus terrestris* L., 1758, *B. lucorum* L., 1761, *B. cryptarum* Fabricius, 1775, *B. magnus* Vogt, 1911 and *B. sporadicus* Nylander, 1848. Two species of this group, *B. lucorum* and particularly *B. terrestris*, are of great economic interest since the extensive use of bumblebees for commercial greenhouse pollination (Velthuis and van Doorn 2006, Winter et al. 2006). In the past decades, there has been much disagreement on the taxonomy of this group. Especially the status of *B. lucorum* and the closely related *B. cryptarum* and *B. magnus*, forming the so-called *Bombus lucorum*-complex, has been intensively discussed. This can be traced to an exceptionally high degree of synonymisation: Williams (1998) reported far more than 100 infrasubspecific names just for *Bombus lucorum* s.l. In contrast, the species status of the three distinct species in Europe is widely accepted nowadays, primarily based on investigations of nucleotide sequences of the mitochondrial COI gene (Bertsch et al. 2005, Murray et al. 2008, Bertsch 2009, Carolan et al. 2012, Williams et al. 2012) and male labial gland secretions (Bertsch 1997,

Bertsch et al. 2004, Bertsch et al. 2005). Still, serious doubts remain concerning the validity of species identification based on morphology and the reliability of certain distinguishing characters have been challenged (e.g., Williams 2000, Carolan et al. 2012). As a consequence of this doubtful delineation, our current knowledge about the species is muddled in a mire of unreliable literature data from numerous authors over the centuries. Only few studies on the species exist that are backed up by reliable species identification using molecular methods. In addition, information about diagnostic characters in the literature are often confusing or based on insufficient underlying data sources. To rectify the problem, this review provides an overview on species recognition and the differentiation of the *Bombus lucorum*-complex. Further, it provides an urgently required reappraisal to pave the way for future investigations.

Bombus lucorum* vs. *Bombus magnus

Bombus lucorum and *B. terrestris* were described by Linnaeus in 1761 and 1758, respectively. Their species status has been widely accepted in the last century. Only few authors doubted their status and lumped them together (e.g., Faester and Hammer 1970, Warncke 1981, Warncke 1986). More than a century later, *B. magnus* was described by Vogt (1911) in a single sentence as a ‘forma nova magnus’ without detailed information. It was probably the same species that was described as *Bombus terrestris* var. *flavoscutellaris* by Trautmann and Trautmann (1915). The species description of *B. magnus* was made by Krüger (1951, 1954) with detailed descriptions of all castes and several *races* and *ethna*, which are difficult to comprehend from today’s view. Some earlier experts failed to distinguish *B. lucorum* and *B. magnus* (Elfving 1960, Ander 1965), others primarily highlighted the need of further studies (Alford 1975, Delmas 1981). Løken (1973) conducted a grand morphometric analysis and advocated their species status, primarily based on measurements of queens, whereas the distinguishability of workers and males remained uncertain. Her work was confirmed and enhanced by further specific indices by Tkalců (1974). At that time, the first biochemical results in the form of male labial gland marking pheromones emerged (Kullenberg et al. 1970, Bergström et al. 1973, Bergström et al. 1981). For *B. lucorum*, two similar but distinctly different profiles could be identified related to a ‘dark’ and a ‘blonde’ form, supporting Løken’s (1973) view. However, common to all of the above mentioned literature is the fact that a previously unknown species, *B. cryptarum*, occurs sympatrically with *B. lucorum* and *B. magnus* and probably biased their results due to a species mix in their samples. This is likely the reason why the results from Pekkarinen (1979) are not in line with the others. Even though other authors also overlooked a possible third taxon (Scholl and Obrecht 1983, Pamilo et al. 1984), their results based on enzyme electrophoresis strongly supported the idea that *B. lucorum* is not a single species.

A third species comes into play

Using morphological and morphometric methods, Rasmont (1981a, 1981b) was the first who recognized a putative third species and attributed it to *Bombus lucocryptarum* Ball which was later synonymized with *Bombus cryptarum* Fabricius (Rasmont 1983). Interestingly this taxon was also previously described as *Bombus lucorum* var. *pseudocryptarum* Skorikov from Russia and Poland (Skorikov 1913). Rasmont (1981b) provided a determination table for the queens. Tables for both female castes (Rasmont 1984) and males (Rasmont et al. 1986) followed, even if those for the latter were of limited applicability. His keys were supported by remarkable crossing experiments between the three putative species (De Jonghe 1982, De Jonghe and Rasmont 1983, Rasmont and De Jonghe 1985). His cross breeding of the three putative taxa ultimately failed, even though copulation and egg deposition were observed. Nonetheless, his breeding within the examined taxa succeeded. In contrast, no interspecific mating was observed in the experiments of Bučánková et al. (2011). In short, the results strongly indicate that reproductive isolation is present but, due to the limited sample sizes, this conclusion cannot be entirely reliable. In general, although the conviction that *B. lucorum* consisted of more than one taxon grew, the species were still lumped together by some authors (Warncke 1986, Westrich 1990). Williams (1991, 1998) provisionally synonymized the potential species. The confirmation of a third species with biochemical methods remained open for some time (Obrecht and Scholl 1984, Scholl et al. 1990, Scholl et al. 1992, Pamilo et al. 1997), probably due to the similar enzyme genetic profiles of *B. cryptarum* and *B. magnus*. However, it is likely that the samples of *B. cryptarum* and *B. magnus* used for analyses were mixed, a point that Bertsch et al. (2004) presupposed for Pamilo et al. (1997). With recurring theme, the morphometric attempts of Baker (1996) were of restricted value, since *B. cryptarum* was not considered as a separate species and the same applies for Macdonald (1999). He advocated *B. lucorum* and *B. magnus* as good species based on the coloration of the pile (extended yellow collar of queens of *B. magnus*; for a review of morphological traits see below) and observations concerning their ecology. In retrospect, it seems highly likely that at least some of the examined specimens from his study were in fact *B. cryptarum*, since this species occurs most frequently in the mainland of northern Scotland (Macdonald, personal communication). This may explain why Williams (2000) could not find a clear gap in collar extension between *B. lucorum* and *B. magnus*: *B. cryptarum* queens have on average a collar extension between the latter two species (Carolan et al. 2012) which may have critically biased the measurements.

The first sufficient biochemical evidence for all three species was conducted by Bertsch (1997) and Bertsch et al. (2004) by the identification of three distinct male labial gland secretion profiles: the profiles of *B. cryptarum* and

B. magnus are similar and share ethyl dodecanoate as the main component. Yet they clearly differ in minor components such as alcohols (Bertsch et al. 2004, Bertsch et al. 2005). Recently the great stability of the labial gland secretion composition of *B. cryptarum* over great geographical ranges was shown, a fact that supports their value for species recognition (Bertsch and Schweer 2012).

Nucleotide sequence data improved our understanding

The debate gained new life with the application of phylogenetic analyses using nucleotide sequences of the mitochondrial cytochrome oxidase I gene (COI). With this method, the composition of three distinct molecular operational taxonomic units (MOTUs) in the European *B. lucorum*-complex was convincingly confirmed multiple times (Bertsch et al. 2005, Murray et al. 2008, Bertsch 2009, Carolan et al. 2012). In contrast, the taxonomic state of knowledge remains incomplete in the global context and additional cryptic taxa of the subgenus *Bombus* s. str. occur in the Far East (Williams et al. 2012). Several Asiatic taxa are most closely related to *B. cryptarum* based on COI (Bertsch et al. 2010) and the difficulty of defining the respective taxonomic units is an ongoing process (Bertsch et al. 2014). Admittedly, although COI barcoding and its applicability for species recognition has been criticized (e.g., Will and Rubinoff 2004, DeSalle et al. 2005, Meyer and Paulay 2005, for a review see Taylor and Harris 2012), the results for the European *B. lucorum*-complex seem convincing. The interspecific genetic divergences of the species are considerably larger than the intraspecific divergences and these patterns are stable over wide geographic ranges of Europe. In measureable terms, the genetic divergences between the species, based on the Kimura 2-parameter model of DNA sequence evolution (Kimura 1980), from Carolan et al. (2012) ranged from 0.033 to 0.044, whereas intraspecific distance was from 0.002 to 0.004. In the analysis by Murray et al. (2008), which was based on Tamura-Nei (Tamura and Nei 1993), the distances are slightly smaller. The interspecific distance ranges from 0.023–0.036 and intraspecific from 0.001–0.004. Based on their divergences and missing intermediates, Murray et al. (2008) concluded that their results “provide strong support for the existence of *B. cryptarum*, *B. lucorum*, *B. magnus* and *B. terrestris* as species that are discrete genotypic clusters” with respect to the Genotypic Cluster Concept of species (Mallet 1995).

Additionally, the COI sequences are suitable for inexpensive and fast analyses by restriction fragment length polymorphisms (RFLP), if only the species identity and not the individual sequence is of interest. Therefore Murray et al. (2008) provided a protocol which was successfully applied by Waters et al. (2011). An enhanced version was published recently (Vesterlund et al. 2014). This more time consuming approach works well with smaller COI fragments and hence is better suited for degraded DNA. However, it should be mentioned that none of the RFLPs protocols worked with the so-called *Folmer re-*

gion PCR primers (derived from the primers presented in Folmer et al. (1994)), which are widely used for DNA ‘barcode’ collections such as BOLD (Ratnasingham and Hebert 2007).

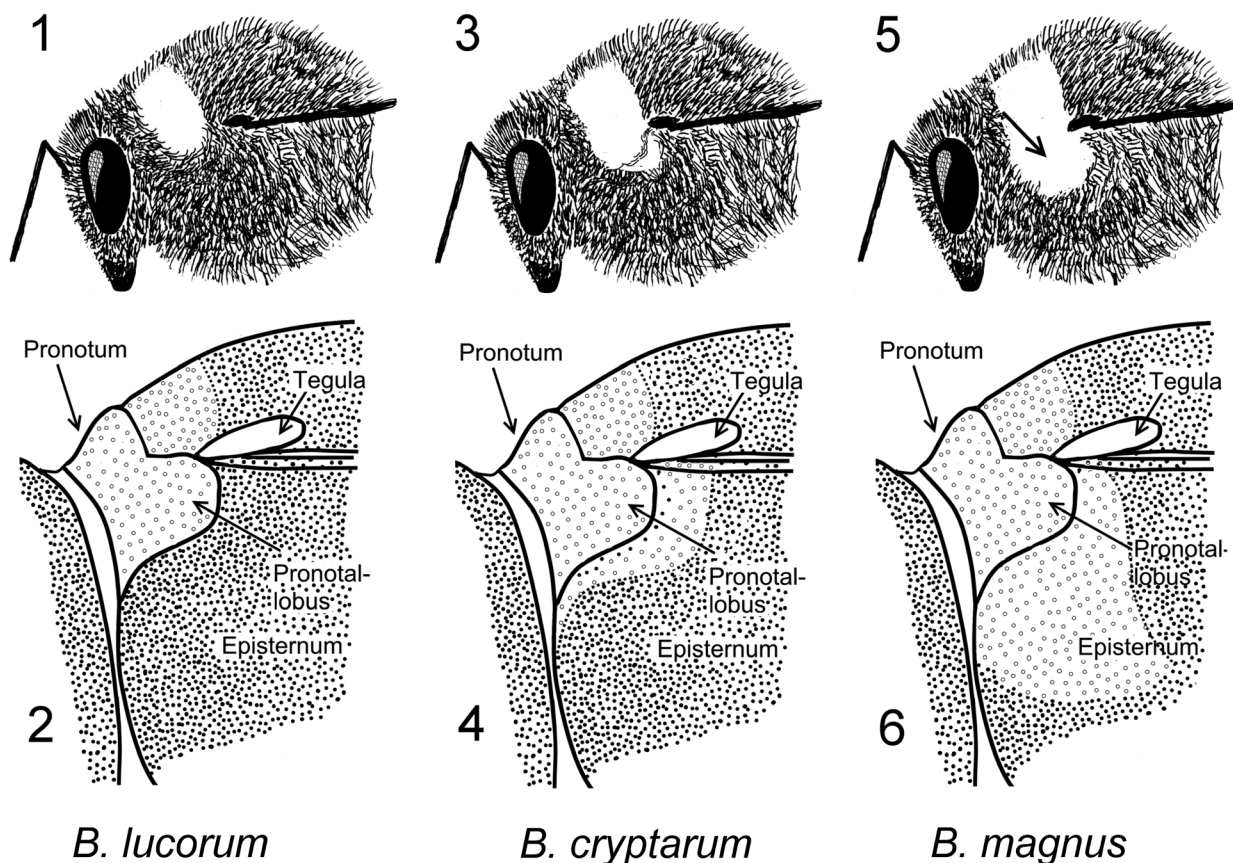
In conclusion, both the labial gland secretion profiles and the results from the analyses of the nucleotide sequences reveal sufficient support for three distinct species in the European *B. lucorum*-complex. Additional support comes from the morphological implications, the phenological data presented in Bertsch et al. (2004) and the cross-breeding experiments. To further enhance our knowledge in this respect, an investigation of nuclear genes of the three species is urgently needed and will be a key issue in understanding the closer phylogenetic relationships in the species complex. In the best case, data from nuclear genes may help clarify the status of the described subspecies of *B. cryptarum* (cf. Rasmont 1984).

Can the species be distinguished by morphology?

While the biochemical and genetic methods for determination are widely accepted today, the published information on the morphological distinguishability of the species is confusing. Fortunately molecular and biochemical methods used nowadays allow for the verification or invalidation of potential discriminatory morphological traits. Currently, the key in Rasmont (1984) is the most important reference for the determination of females since most other keys (e.g., Mauss 1994, Amiet 1996, Bertsch et al. 2004, Dorow 2004) share crucial traits with that of Rasmont or are based on it. In general, the characters of coloration have been examined much more intensively. It should be mentioned that in using Rasmont (1984), the entirety of characters are only recognizable in queens. In this respect, the occurrence of the first collar is particularly important, since this may be the only character that is accessible in the field (Rasmont 1984, Bertsch 1997, Bertsch et al. 2004).

Identification of queens

Three distinct forms of the first collar have been suggested to identify queens from the *B. lucorum*-complex. The first describes the lateral border of the yellow collar, which has been mentioned as a characteristic trait many times (e.g., Skorikov 1913, Ball 1914, Trautmann and Trautmann 1915, Alford 1975, Rasmont 1981b, Rasmont 1984, Amiet 1996, Bertsch 1997, Bertsch et al. 2004). If the border extends down onto the episternum, it is associated with *B. magnus* (Fig. 5) and *B. cryptarum* (Fig. 3). For *B. magnus*, the collar was reported to extend far below the tegulae and become very broad below them. In contrast, a higher lateral border that is almost exclusively restricted to the pronotal lobes points to *B. lucorum* (Figs 1–2). In the literature, this trait is often vaguely described as “below tegula” or not, which is not entirely correct,



Figures 1–6. Shape of first collar as described in the literature. Head and parts of the mesosoma of the respective species are shown in a lateral view. 1–2. *B. lucorum*; 3–4. *B. cryptarum*; 5–6. *B. magnus*. The drawings were provided by Johann Neumayer and are based on Bertsch et al. (2004).

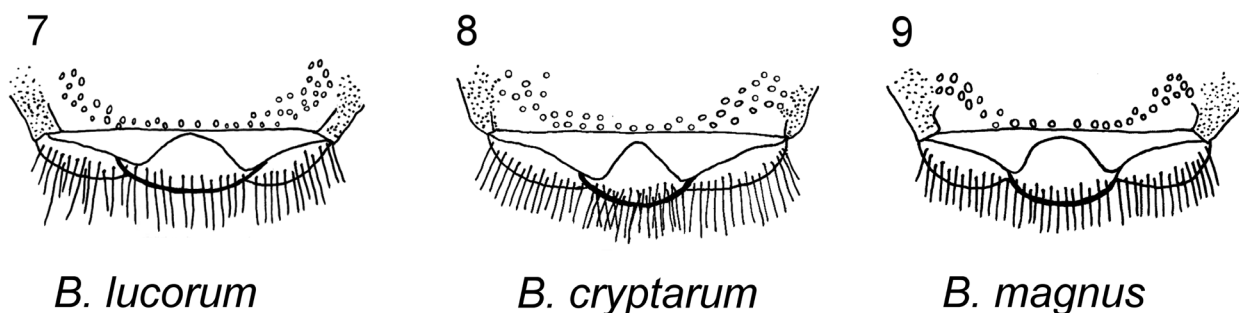
since the border of the episternum is slightly below the tegula. The second trait is a so-called “S” or “5” shape that can be found within the collar. The pile along the border of the pronotal lobes and the episternum may be black and forms the “S” shape (Figs 3–4). It is associated with *B. cryptarum*.

Another hint comes from a strong melanization of the collar which has been reported for *B. cryptarum*. However, this trait is regionally restricted (Bertsch et al. 2004, Bertsch et al. 2005) and on rare occasions may occur in the other species, too (Carolan et al. 2012).

Bertsch (2009) was able to assign all but three investigated queen specimens to the correct species with the above mentioned characters, according to the biochemical evidence ($n = 28$). In contrast, using a larger sample from the British Isles and Denmark ($n = 67$), Carolan et al. (2012) showed that especially the collar-characters are not reliable for species diagnosis since they show overlap (see Fig. 4: doi: 10.1371/journal.pone.0029251.g004). However, not every voucher of this figure is convincing: A close look at specimen “h” from their study, identified by morphology as “*lucorum*”, reveals an obvious collar extension far below the tegula and onto the episternum. Since there is no “S”-shape, the specimen should therefore be associated with *B. magnus*, which is actually the case according to the DNA barcode. More-

over, specimen “c”, which was identified as “*magnus*” based on morphology, reveals a faint black “S”-shape, exactly as described in Bertsch et al. (2004). It remains unclear why this voucher was assigned to *B. magnus* and not *B. cryptarum*. Thirdly, specimen “f” is not a typical *B. magnus*-morphotype since it does not show a clear broad collar below the tegula. Against this background, their conclusion that “each species can be morphologically identified as belonging to all 3 taxa” cannot be upheld. The study gave sufficient evidence that the extension of the anterior band of *B. cryptarum* queens can vary and that it critically resembles the traits of the other species. Yet it does not show that queens of *B. lucorum* and *B. magnus* resemble each other.

Aside from this confusing information, the work of Carolan et al. (2012) strongly indicated that the collar characters are not completely reliable and should not be exclusively taken into account for species identification. In addition, the key of Rasmont (1984) uses several characters aside from the coloration of the pile, such as the form of the labrum, punctuations of several structures and the shape of the hindleg metatarsus. However, the reliability of these characters has not been examined against independently verified specimens and through broad geographic sampling. Thus the identification of queens of the *B. lucorum*-complex cannot be made by morphological



Figures 7–9. Labrum characters in frontal view. 7. *B. lucorum*; 8. *B. cryptarum* 9. *B. magnus*. The drawings were provided by Johann Neumayer and are based on Rasmont (1984).

traits with complete certainty, even if most specimens are probably easily determined as described previously (Bertsch 1997, Bertsch et al. 2004, Bertsch et al. 2005).

Identification of workers

The current state of knowledge concerning the identification of workers is worse than that for queens. Rasmont (1984) postulated that the coloration of workers corresponds approximately to the coloration of queens, implying a potential distinguishability in the shape and extension of the first collar. Unfortunately, the “S”-shape of *B. cryptarum* workers can be inconspicuous or absent (P. Rasmont, personal communication). Indications for the recognition of *B. magnus* can arise if yellow hair is mixed in the black pile of the first tergum (Rasmont 1984). Recently, the distinguishability of the anterior yellow band was examined quantitatively with Scottish (Waters et al. 2011) and Austrian specimens (Bardakji 2013) and was verified with RFLPs and DNA barcodes, respectively. Both studies revealed an uncertain connection of the traits to the species. In Scotland, where all three species occur sympatrically, Waters et al. (2011) was unable to properly distinguish *B. cryptarum* from the other two species. Still there was significant difference in collar extension between *B. lucorum* and *B. magnus*. It seems that the collar extension of the Scottish *B. cryptarum* is moderately variable and therefore constrains the possibility to recognize the other two species. Unfortunately, data on the pile coloration of the first tergum were not recorded, therefore the accuracy of this potential character remains uncertain.

In a study with Austrian specimens (Bardakji 2013), the sample consisted of *B. lucorum* and *B. cryptarum* individuals only. Regarding the extension of the collar, Bardakji (2013) was able to identify a great part (85.5%, 47 of $n = 55$) of the workers correctly. There were considerably more identification errors in *B. cryptarum*, supporting the view that the extension of the collar of workers of *B. cryptarum* is more variable, in contrast to the others species. Aside from coloration, Rasmont (1984) described two groups of morphological characters that are accessible in queens and workers. (I) The first distinguishes characters of the labrum, e. g., the form of the basal area, especially if it is “U”-shaped (*B. lucorum* and *B. magnus*, Figs 7 and 9, respectively) or “V”-shaped (*B.*

cryptarum, Fig. 8). Further, the form of the lamella and punctuation are additional characters of potential value. (II) The second group describes the punctuations of the second tergum. Based on this, it was possible only to distinguish *B. lucorum* but not *B. cryptarum* or *B. magnus*. In contrast, Bardakji (2013) tested the reliability of the tergum-trait to differentiate between *B. lucorum* and *B. cryptarum*. It failed in roughly 1 of 5 cases. This is in line with the view of Dorow (2004), who challenged this character by describing greater variation of the second tergum than previously described (Rasmont 1984). In any case, as mentioned above, *B. magnus* was not present in the sample used by Bardakji (2013) and therefore no general statements can be made. Still, it is strongly advised to test these traits on a larger scale with all three species. To avoid misunderstandings it is important to separately name the essential structures. The lamella is the structure directly below the basal area of the labrum and is neither “U” nor “V”-shaped. These shapes refer instead to the basal elevation of the labrum (Figs 7–9).

In summary, the possibility to identify workers of all three species based on morphology has not been verified. Nonetheless, the characters of the labrum and the second tergum are particularly promising. Further morphological comparative examinations, which are supported by DNA barcoding, are necessary to verify these potential identifying characters and to uncover new traits. In the field, the extension of the first collar may be an indicator but is definitely not reliable, especially if all three species co-occur. Additionally, the reliability of the yellowish coloration of the first tergum for workers of *B. magnus* is worthy of further investigation.

Identification of males

Identification of the males is probably the most difficult case. Authors of recently published studies agreed that they are indistinguishable by morphology (Murray et al. 2008, Bertsch 2009, Waters et al. 2011). All three taxa are very similar and show extensive and overlapping variation in color and male genitalia (Rasmont et al. 1986). Therefore, keys based on coloration of the pile of the “face” (e.g., Amiet 1996, Dorow 2004, Gokcezade et al. 2010) are of restricted value, even if they may work for certain geographic regions. In the wider European context, these

keys will fail to properly distinguish all male specimens of the complex. Aggravating this situation is the fact that the males of *B. terrestris* may also be confused with males of the *B. lucorum*-complex, in particular with specimens of *B. cryptarum* that have a dark facial pile. Although, *B. cryptarum* males often show the “S”-shape, it is geographically restricted and especially *B. cryptarum* and *B. magnus* can be more or less identical in morphology (P. Rasmont, personal communication). Aside from coloration, Rasmont et al. (1986) described several potential morphological characters to identify males at the species level. In this respect, the authors highlighted the punctuation of the second tergum as a distinguishing character for *B. lucorum* against *B. cryptarum* and *B. magnus*. Additional characters concern the diameter of the ocelli and the shape of the eighth tergum. The reliability of these traits in the wider European context remains uncertain. As long as no new insights in the distinguishability of the males are gained, completely reliable identification can only be achieved by biochemical or genetic approaches.

Current challenges

Difficulty in species recognition constrains our current knowledge

The long and difficult process of the recognition and acceptance of the species of the *B. lucorum*-complex has caused a number of critical problems concerning our current knowledge of the ecology and distribution of these species. First, the lack of applicable characters that are useful and easy for identification makes it difficult to obtain reliable data from the literature. The great majority of previous studies on these species are based on morphological identification and hence should be viewed cautiously. Additionally, the possibilities of achieving faunistic data by interested amateurs and citizen scientists are very restricted and can barely contribute to scientifically founded statements in this concern. Second, the late re-description of *B. cryptarum* by Rasmont (1981a) implies that practically all data published before the re-description are unreliable since it was not possible to distinguish the species based on the debatable morphological traits. An example from Austria illustrates this point. All reported findings of *B. magnus* from Austria known to the author either before or shortly after the re-description, including the records of Schedl (1982) and Mathis (1982), findings from W. F. Reinig in Aistleitner (2000) and Ressler (1995), and the findings from B. Tkalců in Neumayer and Kofler (2005), were recently reexamined and found to belong to *B. cryptarum* based on morphology (J. Neumayer, personal communication). This demonstrates the importance of verifying older records from the literature and reveals that the unconditional use of references published before that date could lead to confusion, such as the citation of the textbook of Alford (1975) in Murray et al. (2008) or Waters et al. (2011). Third, the predicament is addition-

ally aggravated by the treatment of *B. cryptarum* by authors. Several experts declined to immediately accept *B. cryptarum* as a valid taxon and pooled the available data. In a strict sense, the identification method of every contribution should be reexamined, and the information in several reference textbooks or compilations (e.g., Prŷs-Jones and Corbet 1987, Westrich 1990) unfortunately cannot be regarded as totally reliable. In light of these problems, the number of dependable studies is much reduced. Reliable ecological and distributional data is primarily available in recent studies based on biochemical identification methods. Further, the excellent publications of Pierre Rasmont (Rasmont 1981a, Rasmont 1983, Rasmont 1984, Rasmont et al. 1986, Banaszak and Rasmont 1995) deserve our continued attention regarding the bumblebees of the *B. lucorum*-complex.

Current issues concerning the distribution and ecology

Bertsch et al. (2004) carefully outlined the distribution of the species. Additional data comes from the recent COI-based studies (Murray et al. 2008, Anagnostopoulos 2009, Bertsch 2009, Waters et al. 2011, Carolan et al. 2012, Vesterlund et al. 2014) and from the distribution maps on bumblebees in the Atlas Hymenoptera project (Rasmont and Pauly 2010, Rasmont and Iserbyt 2010–2013). In these works the distribution, especially of *B. cryptarum* and *B. magnus*, appears fragmentary. The isolated finding of *B. cryptarum* in the Balkans (Anagnostopoulos 2009), the lack of doubtless identified *B. magnus* from the Iberian Peninsula south of the Pyrenees and the old records from Eastern Europe reveals the need of further sampling in these regions. Specimens from the Iberian Peninsula are of particular importance since there are indications that queens of *B. lucorum* exhibit a collar coloration similar to *B. magnus* queens in central Spain (Bertsch 2009). Against the background of the false records from the Austrian Alps, the presence of *B. magnus* in the Western Alps and along the southern slopes of the Alps must be verified as well. Species identification accomplished using COI barcodes should contribute to clarify our patchy knowledge on the distribution of the genetic haplotypes and might help outline postglacial recolonization events.

Further investigations are also necessary to understand the factors that drive the species abundances on regional and European scales, since species composition can vary greatly at the regional level (Murray et al. 2008, Waters et al. 2011). One factor that has been claimed to influence the species composition is altitude. For example, Murray et al. (2008) revealed a changing species composition along a relatively low altitudinal gradient, and Neumayer and Paulus (1999) regarded *B. cryptarum* to be a high mountain species. Further, Scholl and Obrecht (1983) concluded that one *B. lucorum* s.l. taxon occurs at higher relative frequency in the Alps. In contrast, the fact that all species can be found to live sympatrically in various lowland habitats in greater parts of Europe means that altitude cannot be the determining factor for a species' distribu-

tion. However, the association of *B. cryptarum* with the high altitudes of the Alps and the observation of Pamilo et al. (1997) that *B. cryptarum*/*B. magnus* becomes predominant in northernmost Finland justify further investigation into the ecological factors that change with increasing altitude and latitude. Additional research is still pending concerning habitat use and nesting biology. Waters et al. (2011) made significant inroads to understand habitat usage but the study was restricted to relatively few habitats in northwestern Scotland. Regarding continental Europe, most recent studies specify the habitat types of the collection areas, but comparative studies concerning the used habitat or nesting sites over a sufficient geographic area are not available. Especially the exact habitat use of *B. magnus* appears unclear. The occurrence of this species seems to be very patchy but regionally common (Bertsch et al. 2004). Further, it is frequently associated with heathlands (Banaszak and Rasmont 1994, Waters et al. 2011) and visits species of Ericaceae (Rasmont 1984, Bertsch et al. 2004). Yet, the species is neither restricted to heathland nor does it rely on Ericaceae. Of particular note is that the species seems to occur in habitats with a very low diversity of flowering plant species, such as mass-flowering Ericaceae in heathlands or *Melampyrum pratense* in commercial forests (personal observation). Comparative studies are also necessary to improve our knowledge of the species bionomies, e.g., by examining exact nesting sites, and might confirm the phenological differences suggested by Bertsch et al. (2004).

The importance of regionally stable characters

Traditionally, a significant part of the faunistic data of bumblebees in Europe is contributed by dedicated amateurs from the public rather than institutional scientists. At present, the restrictive possibility of identifying specimens by morphology has prevented reports of species of the *Bombus lucorum*-complex by citizen science. However, observations described in the literature suggest that species of the complex exhibit certain characters in certain regions such as the characteristic melanized collar, probably restricted to queens of *B. cryptarum* in northern Germany (Bertsch et al. 2005), or the “pinkish-buff” on the metasoma of fresh *B. magnus* queens that was reported from Northern Scotland (Macdonald 1999). It should be worthwhile to consider the reliability and stability of such characters to allow the public to make use of them for morphologically based identifications in particular regions. In this respect, particularly promising is the coloration of clypeal hairs in males. Admittedly males show extensive color variation in facial hair (Rasmont et al. 1986, Table 2), yet there are indications that regionally stable characters exist. The keys of Amiet (1996), Dorow (2004) and Gokcezade et al. (2010) share the same color-based system to distinguish males of all species from Switzerland, Hesse (Germany) and Austria, respectively. An examination of the reliability of these traits in the mentioned regions is of particular value, since the rele-

vant characters are accessible in the field and hence might serve as a window to achieve distributional data without the need for molecular or biochemical analyses.

Future tasks

1. Investigations of nuclear genes of the species from the *Bombus lucorum*-complex will contribute to underpin the species' status and help to estimate more accurate phylogenies.
2. New genetic sequence data, especially from the Mediterranean peninsulas, will enhance the current knowledge about the genetic diversity within the complex and might help to evaluate potential postglacial recolonization events.
3. The reported distinguishing morphological characters must be tested for all castes of all species in a wider European context against a biochemical control and on a sufficiently large scale. Further, it would be of particular importance to discover new distinguishing characters.
4. Investigations to determine regionally stable morphological or coloration characters might facilitate the acquisition of new distributional and ecological data by citizen scientists.
5. The reexamination of museum specimens, at best, backed up with a sequencing of COI fragments, can allow the correct assignment of historic records and will help to highlight incorrect species identifications.
6. Additional acquisition of good ecological and distributional data from accurately identified specimens will increase our knowledge about the species' ecology. Among others, future studies should focus on altitudinal differences, nesting sites and habitat use of the species.

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