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Unveiling cryptic species of the bumblebee subgenus *Bombus* s. str. worldwide with COI barcodes (Hymenoptera: Apidae)

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Research Article

Unveiling cryptic species of the bumblebee subgenus *Bombus s. str.* worldwide with COI barcodes (Hymenoptera: Apidae)

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Bumblebees of the subgenus *Bombus s. str.* dominate (or used to dominate) many north temperate pollinator assemblages and include most of the commercial bumblebee pollinator species. Several species are now in serious decline, so conservationists need to know precisely which ones are involved. The problem is that many *Bombus s. str.* species are cryptic, so that species identification from morphology may be impossible for some individuals and is frequently misleading according to recent molecular studies. This is the first review of the entire subgenus to: (1) avoid fixed a priori assumptions concerning the limits of the problematic species; and (2) sample multiple sites from across the entire geographic ranges of all of the principal named taxa worldwide; and (3) fit an explicit model for how characters change within an evolutionary framework; and (4) apply explicit and consistent criteria within this evolutionary framework for recognising species. We analyse easily-obtained DNA (COI-barcode) data for 559 sequences from 279 localities in 33 countries using general mixed Yule-coalescent (GMYC) models, assuming only the morphologically distinctive species *B. affinis* Cresson, *B. franklini* (Frison), *B. ignitus* Smith and *B. tunicatus* Smith, and then recognise other comparable COI-barcode groups as putative species. These species correspond to modified concepts of the taxa *B. cryptarum* (Fabricius), *B. hypocrita* Pérez, *B. jacobsoni* Skorikov, *B. lantschouensis* Vogt **n. stat.**, *B. longipennis* Friese, *B. lucorum* (Linnaeus), *B. magnus* Vogt, *B. minshanensis* Bischoff **n. stat.**, *B. occidentalis* Greene, *B. patagiatus* Nylander, *B. sporadicus* Nylander, *B. terrestris* (Linnaeus) and *B. terricola* Kirby (a total of 17 species). Seven lectotypes are designated. Our results allow us for the first time to diagnose all of the putative species throughout their global ranges and to map the extent of these geographic ranges.

Key words: barcoding, *Bombus*, bumblebees, *lucorum*-complex, pollinators, systematics, taxonomy

Introduction

Almost all of the bumblebee species that are used commercially for the pollination of glasshouse crops around

the world, an industry worth billions of dollars annually (Dias *et al.*, 1999; Winter *et al.*, 2006; Goulson, 2010), belong to the subgenus *Bombus s. str.* (a monophyletic group: Williams, 1995; Cameron *et al.*, 2007). One species, *Bombus* (*Bombus*) *terrestris* (details of taxon authors are in the Appendix), has been especially popular for glasshouse

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pollination and has been introduced into several countries where it is not indigenous, including New Zealand, Chile and Japan (Goulson, 2003; Winter *et al.*, 2006). Unfortunately, it now appears that there are serious risks associated with moving bumblebees between countries, even for glasshouse pollination. For example in Japan, *B. terrestris* was introduced into glasshouses, but feral colonies were soon discovered and the species is spreading and replacing the indigenous *B. hypocrita* (Matsumura *et al.*, 2004; Inoue *et al.*, 2008). In North America, there is circumstantial evidence that using bumblebees reared in Europe may have caused the rapid and severe collapse of populations of the native *B. affinis*, *B. terricola* and *B. occidentalis*, which were previously among the most abundant species (Thorp, 2003; Winter *et al.*, 2006; Colla & Packer, 2008; Cameron *et al.*, 2011, but see Brown, 2011). In order to avoid possible similar collapses, commercial growers in other areas outside Europe now want to know which closely related indigenous species they have that might also perform well as pollinators in glasshouses (Williams *et al.*, 2010). The problem is that the taxonomy of the species within this subgenus has been particularly uncertain, especially in Asia. There have been no recent revisions (or keys) for the entire group, and just two lists of the world fauna, by Skorikov ([1923]: 19 species) and Williams (1998: 10 species, but including an unresolved complex of several cryptic species). Only a few of the species are easily diagnosed morphologically and widely agreed upon, so that there remains a great morass of taxa of uncertain status and affinity (with 408 published names). These taxa have only subtly differentiated and variable morphological characters from which to diagnose them. Consequently, both a sustainable global pollination industry and the conservation of biodiversity and ecosystem services urgently require clarification and accurate identification of the species of *Bombus s. str.*

Cryptic species are those that satisfy an accepted concept of species, but which are closely similar or identical in morphology. Identifying species of the subgenus *Bombus s. str.* from morphology has always presented greater problems than in many other groups of bumblebees. Frustratingly, the relatively close scrutiny that this small group has attracted from many authors, especially in Europe, has not resulted in these problems being resolved. For example, in western Europe until the mid-twentieth century only two species (*B. terrestris* and *B. lucorum*) were widely accepted (Pittioni, 1939). Even these two are still considered difficult to distinguish (Delmas, 1976; Wolf *et al.*, 2010). When Krüger (1951, 1954, 1956, 1958) reviewed colour variation within the group in considerable detail, he recognised *B. magnus* as a third west-European species. *Bombus magnus* became more widely accepted after other bumblebee taxonomic specialists, Løken (1973) and Tkalcu (1974b), also recognised it as a separate species. But then another taxon that could not immediately be named was identified among males of '*B. lucorum*' from labial gland secretions

(Kullenberg *et al.*, 1970; Bergström *et al.*, 1973). Subsequently Rasmont (1981, 1983, 1984) argued that *B. cryptarum* was a species long-ignored in Europe. The three most similar-looking major taxa (*B. lucorum*, *B. cryptarum*, *B. magnus*, henceforth the 'LCM' taxa) came to be regarded as a 'species complex' (Delmas, 1981; Scholl & Obrecht, 1983), or as a group of 'sibling species' (Pamilo *et al.*, 1984), although other experienced specialists disagreed with recognising all three as separate species (Løken, 1966; Tkalcu, 1969; Pekkarinen, 1979). Not only was it found that not all individuals could be identified with confidence among the three LCM taxa using morphological characters (Rasmont, 1984; P. Rasmont, pers. comm.), but specialists also disagreed among themselves on the precise thresholds (along an apparent continuum of morphological variation) for diagnosing them (Williams, 2000). Consequently, as an interim pragmatic measure, the LCM taxa have sometimes been treated as an unresolved complex (Williams *et al.*, 2009), until it becomes possible to recognise and diagnose the species reliably, with the aim of minimising the inevitable but misleading misidentifications. Meanwhile, support for the interpretation that the LCM taxa do indeed correspond to three separate species has grown from studies of morphology (Rasmont *et al.*, 1986), enzyme electrophoresis (Scholl & Obrecht, 1983; Pamilo *et al.*, 1984), male labial gland secretions (Pamilo *et al.*, 1997; Bertsch *et al.*, 2004), and DNA sequences (Pedersen, 2002; Bertsch *et al.*, 2005; Murray *et al.*, 2008; Waters *et al.*, 2010). Unfortunately though, the situation is still not entirely resolved. Despite a recent claim that in western Europe the LCM taxa constitute three separate species with both characteristic colour patterns of the pile and characteristic COI (DNA) sequences (Bertsch, 2009), a larger study has shown that the colour-pattern groups and the DNA groups frequently disagree (Carolan *et al.*, 2012). Therefore, the LCM taxa appear to be truly cryptic species. Studies of the more diverse species of this group in Asia have been based on widely scattered, small samples that are also difficult to diagnose from morphology (e.g. Rasmont *et al.*, 1986; Williams, 1991; Williams *et al.*, 2009; Bertsch, 2010; Bertsch *et al.*, 2010a), so that crucially all of the European work has been done against a background of a very patchy knowledge of its larger global context.

Substantial progress towards resolving cryptic species within the subgenus *Bombus s. str.* is likely to require more characters in addition to morphology. DNA sequences provide a particularly rich source of characters and one for which models describing patterns of evolutionary change are available. Fortunately, recent pinned specimens can be used to recover the short 'barcode' sequence of the mitochondrial COI (*cox-1*) gene that has been found to enable discovery of species identical to those recognised by more traditional methods (Monaghan *et al.*, 2009). Inheritance of mitochondrial genes such as COI is reasonably well understood (Ballard & Whitlock, 2004). Using these genes avoids

the problems with nuclear genes that arise from having multiple alleles, while absence of indels makes alignment for homology straightforward. Mitochondrial genes also have a relatively high substitution rate, so it is claimed that even the short COI-barcode region of 658 nucleotides can be used to identify the most closely related taxa (Hebert *et al.*, 2003; Baker *et al.*, 2009), and indeed COI-barcode sequences as short as 100 nucleotides can be diagnostic for 90% of the species in other animal groups (Meusnier *et al.*, 2008). Even when seeking to infer phylogeny, greatly increasing the length of the mitochondrial genome sequences used does not improve the results, contrary to expectation (Naylor & Brown, 1997). Consequently, despite many potential pitfalls and the need for external supporting evidence, COI barcodes can still be useful for recognising likely cryptic species (Brower, 2006). And encouragingly, a survey of the subgenus *Subterraneobombus* worldwide (Williams *et al.*, 2011) has demonstrated that COI barcodes can recover conventional, morphologically diagnosable species for bumblebees, while not splitting these into many false, supposedly 'cryptic species'. Obtaining COI-barcode data is now widely available as a low-cost service, with a campaign already in place specifically to facilitate the barcoding of bees worldwide (Packer *et al.*, 2009).

The aim of this paper is to provide an overview of COI-barcode variation within the subgenus *Bombus s. str.* worldwide, from which to recognise and diagnose species. The contributions of the authors have been as follows: PW, collecting material, databasing, data analysis, nomenclature, and manuscript preparation; MB, JC, DG discussion of analysis and manuscript; JA, JH organising and executing extensive collecting surveys in China; JC, JH, CS providing additional sequences; other authors, collecting material for this study to provide permanent vouchers (deposited in the BMNH).

Materials and methods

Our strategy was: (1) to sample specimens across the global range of the subgenus *Bombus s. str.*, to include the principal currently named taxa and the morphological variation within these taxa; (2) to derive COI-barcode sequences from the specimens; (3) to infer monophyletic groups from the sequence data by fitting an explicit evolutionary model of character change; (4) to recognise putative species among the groups by using explicit, consistent and accountable criteria; and (5) to associate these putative species with type specimens and hence with published names.

Sampling bees

Sample specimens were diagnosed as belonging to the subgenus *Bombus s. str.* from morphological characters in the subgeneric keys in Williams *et al.* (2008). The samples for

Table 1. Abbreviations for depositories from which material has been examined.

| Abbreviation | Address |
|--------------|---|
| ANSP | Academy of Natural Sciences, Philadelphia, USA |
| BMNH | Natural History Museum, London, UK |
| IAB | Institute of Apiculture, Chinese Academy of Agricultural Sciences, Beijing, PRC |
| IBSV | Institute of Biology and Soil Science of the Russian Academy of Sciences, Vladivostok, Russia |
| INHS | Illinois Natural History Survey, Champaign, USA |
| IZB | Institute of Zoology, Chinese Academy of Sciences, Beijing, PRC |
| KU | Christian-Albrechts-Universität, Kiel, Germany |
| KUK | Kyushu University, Kyushu, Japan |
| LP | Prof. Laurence Packer, University of York, Toronto, Ontario, Canada |
| LSL | Linnean Society, London, UK |
| MI | Dr Masao Ito, Sapporo, Japan |
| MNHN | Muséum National d'Histoire Naturelle, Paris, France |
| MNHU | Museum für Naturkunde an der Humboldt-Universität, Berlin, Germany |
| NHR | Natur Historiska Riksmuseet, Stockholm, Sweden |
| NMS | Naturmuseum Senckenberg, Frankfurt, Germany |
| OLL | Oberösterreichs Landesmuseum, Linz, Austria |
| UKK | University of Kansas, Kansas, USA |
| USNM | US National Museum of Natural History, Washington DC, USA |
| ZISP | Zoological Institute of the Russian Academy of Sciences, St Petersburg, Russia |
| ZMA | Zoologisch Museum der Universiteit van Amsterdam, Amsterdam, the Netherlands |
| ZMH | Zoological Museum of the University of Helsinki, Helsinki, Finland |
| ZMMU | Zoological Museum of the Moscow State University, Moscow, Russia |
| ZSM | Zoologische Staatssammlung, München, Germany |

sequencing were selected to include most of the known variation as represented by the previously described specific and subspecific taxa by selecting 'proxy types' for our analysis (see Appendix). Where possible this included individuals from across the entire breadth of the geographic ranges around the Holarctic and Oriental Regions of the principal nominal taxa. In order to achieve a more representative sampling of this large area, we started by exploiting the broad cover of existing museum collections (Table 1) and then used a collaborative network to obtain strategic new samples. Emphasis was placed on reviewing particularly poorly known taxa from Asia, with material included from recent extensive surveys by the authors from China, Central Asia and Russia, supported by GPS data (An *et al.*, 2008, 2010; Williams *et al.*, 2010). Most specimens had been collected within the last 12 years. All material was identified or confirmed to taxa by one author (PW) in order to minimise inconsistencies in the identifications. Specimens with COI barcodes were given unique database identifiers (referred to

here by B#) and data were recorded in an Access database (version 2003 SP3).

DNA-barcode data

Sample specimens were sequenced for the short 5' barcode region of the mitochondrially encoded COI (cytochrome *c* oxidase, subunit 1) gene. The majority of specimens were extracted, amplified and sequenced at the Biodiversity Institute of Ontario, University of Guelph, as part of the BEE-BOL campaign to barcode the bees of the world (Packer *et al.*, 2009). COI-barcode extraction, amplification and sequencing used the standard protocols described by Hebert *et al.* (2003). Universal primers for the COI-barcode sequence for insects were used (variants *LepF1* and *LepR1*; Hebert *et al.*, 2004a). Data for the specimens processed at Guelph have been uploaded to the BOLD online database (Ratnasingham & Hebert, 2007). A minority of specimens were processed at Trinity College, Dublin (JC) as described by Carolan *et al.* (2012), or at the Institute of Apiculture, Beijing (JH). COI-barcode sequences (without primer sequences) from the samples were aligned using the ClustalW function within BioEdit (version 7.0.9.0; www.mbio.ncsu.edu/BioEdit/bioedit.html, accessed 2010) and trimmed to a common frame length of 658 nucleotides that is shared by most samples.

Recognising species

There is no universally accepted concept of the nature of species, or any universally accepted method or criterion for species definition or recognition in practice (Mallet, 1995, 1997). Nonetheless, a few species of the subgenus *Bombus s. str.* (*B. affinis*, *B. franklini*, *B. ignitus* and *B. tunicatus*) can be considered a priori to be broadly accepted as separate and distinctive species (Tkalcu, 1962, 1967, 1974a; Løken, 1973; Plowright & Stephen, 1980; Williams, 1991; Thorp & Shepherd, 2005; Tokoro *et al.*, 2010) that are relatively easily recognisable by pile colour pattern and morphology. The problem lies with assessing the status of the many other described taxa in this subgenus, among which cryptic species are suspected, but for which diagnostic morphological character states are unknown, or incompletely known, or unreliable.

Some previous approaches to recognising species have been based on patterns of relationship within phylogenetic trees (Mallet, 1995, 1997). These approaches examine the branching patterns associated with accepted species and then seek to identify other comparable groups from across a phylogenetic tree as candidates for taxa at the rank of species, if they show similar patterns in terms of tree topology alone. Early analysis of COI barcodes suggested a substantial 'gap' between inter- and intraspecific levels of divergence, which could then be used to recognise whether groups were separate species (Hebert *et al.*, 2004b). How-

ever, this potential universal standard gap has subsequently been found to be unreliable (Meyer & Paulay, 2005; Meier *et al.*, 2006; Schmidt & Sperling, 2008) and is not accepted here. Instead, we look for expected transitions in tree-branching patterns between long interspecific branches and short intraspecific branches by fitting general mixed Yule-coalescent (GMYC) models (Monaghan *et al.*, 2005; Pons *et al.*, 2006; Papadopoulou *et al.*, 2008). Models of these processes for our particular data can be fitted either to apply a single uniform threshold across an entire tree, or to apply multiple thresholds at different heights in different parts of a tree (Monaghan *et al.*, 2009).

GMYC analysis requires an ultrametric estimate of the phylogenetic tree for the unique haplotypes. Duplicate haplotypes need to be removed from the tree, because otherwise nodes with zero 'age' between identical sequences would imply infinite branching rates (T. Barraclough, pers. comm.). We reduced the dataset to unique haplotypes using Collapse (version 1.2; darwin.uvigo.es/software/collapse.html, accessed 2011) after sorting sequences by decreasing length (to avoid matching of longer to shorter sequences, which might obscure real differences). To infer phylogenetic relationships among the unique haplotypes, we used Bayesian analysis. We found that one of the nucleotide-substitution models that fitted our COI-barcode data best according to jModelTest (version 0.1.1; Posada, 2008) is the frequently used general time-reversible model with a gamma frequency distribution of changes among sites and allowing invariant sites (GTR+G+I). BEAUti was used to prepare XML files for submission to BEAST (both of version 1.6.2; beast.bio.ed.ac.uk, accessed 2011) for Bayesian analysis to group samples into trees (Drummond & Rambaut, 2007). The number of gamma categories was set to 6, the clock model was set to the uncorrelated lognormal (fixed rate 1), the tree speciation model was set to a Yule process, and the chain length was set to 40 million generations for the Markov-Chain Monte Carlo (MCMC) algorithm, with sampling of the trees every 4000 generations. The ingroup was selected as a first monophyletic taxon set and the outgroup (used to root the trees) was selected as a second taxon set (not monophyletic). The outgroups were chosen to represent the sister groups of *Bombus s. str.*, using sequences from *B. (Pyrobombus) vagans* Smith, *B. (Alpinobombus) alpinus* (L.), and *B. (Al.) balteatus* Dahlbom. This follows the results of Cameron *et al.* (2007) from a phylogenetic analysis of five genes across most bumblebee species. A consensus tree was obtained from the post burn-in tree sample (rejecting the first 10%) using TreeAnnotator (version 1.6.2; as for BEAST). The posterior probability threshold was set to 0.5, the target tree type was set to maximum clade credibility, and the heights of the nodes were kept.

We applied GMYC models to the ultrametric tree using the SPLITS software tools (T. Ezard; r-forge.r-project.org/projects/splits/, accessed 2011). These tools run

on the platform of the R project for statistical computing (version 2.12.1; www.r-project.org, accessed 2011). The results of the models were assessed in part by examining their consequences for the calibration set of the four broadly accepted species. There are questions concerning the ‘limits’ of all of the other species, which we seek to address. In addition, MacClade (version 4.08, Maddison & Maddison, 2001) was used to fit sequence data to the tree in order to identify diagnostic nucleotide changes for each putative species. MEGA (version 4.0; Tamura *et al.*, 2007) was used to measure intra- and interspecific sequence divergences, which were calculated using the Kimura two-parameter divergence model (K2P, Kimura, 1980). Although poorly justified for barcodes (Srivathsan & Meier, 2011), using the K2P model permits comparison with divergence measures from previous studies of the barcoding ‘gap’.

Naming species

An unsolved problem ignored in most molecular studies is how to associate available formal names (in the sense of ICZN, 1999) with groups of COI-barcode samples on trees, because it is rarely possible to sequence the name-bearing type specimens directly. The problems are that type specimens are: (1) often regarded as too valuable for even small body parts to be removed for destructive sampling; and (2) often too old for simple extraction and sequencing, with degradation-associated ‘lesions’ in their COI sequence (Bertsch, 2009). The association of name-bearing type specimens with COI-barcode groups therefore usually has to be subjective and based on agreement in morphological characters.

We have attempted to associate the original name-bearing types with COI barcodes via informal vouchered proxy types, for which we do have COI barcodes. These proxies are intended for use in this project alone, to make the process of associating names explicit, accountable, and open to easy revision. In some cases the subjective association can be made easily because a named taxon has a distinctive pile colour pattern or morphology and a well characterised distribution, although in other cases the arguments are less certain (see the Appendix for individual details). Where possible, we have obtained recent samples with matching morphology from sites close to the taxon’s original type locality. Names were then associated with the species identified by the GMYC analysis of the unique haplotypes via the sequences of the proxy types by adding accepted sequences back into the haplotype tree. Data files are available from PW.

Results

Quality control of sequence data

A total of 613 specimens were sent for extraction, yielding 572 COI-barcode sequences. No amplification of *Wolbachia* (or other obviously non-target DNA) was detected. A few samples read differently in the forward and reverse directions, or sequences had grouped basally as outliers within the tree (with no geographic or morphological association, and not as misidentifications according to searches using the BOLD identification engine), or are very short. These are interpreted as severely degraded and are rejected. The remaining 558 sequences (91% of specimens sampled) from 279 localities (Fig. 1, plus one sequence

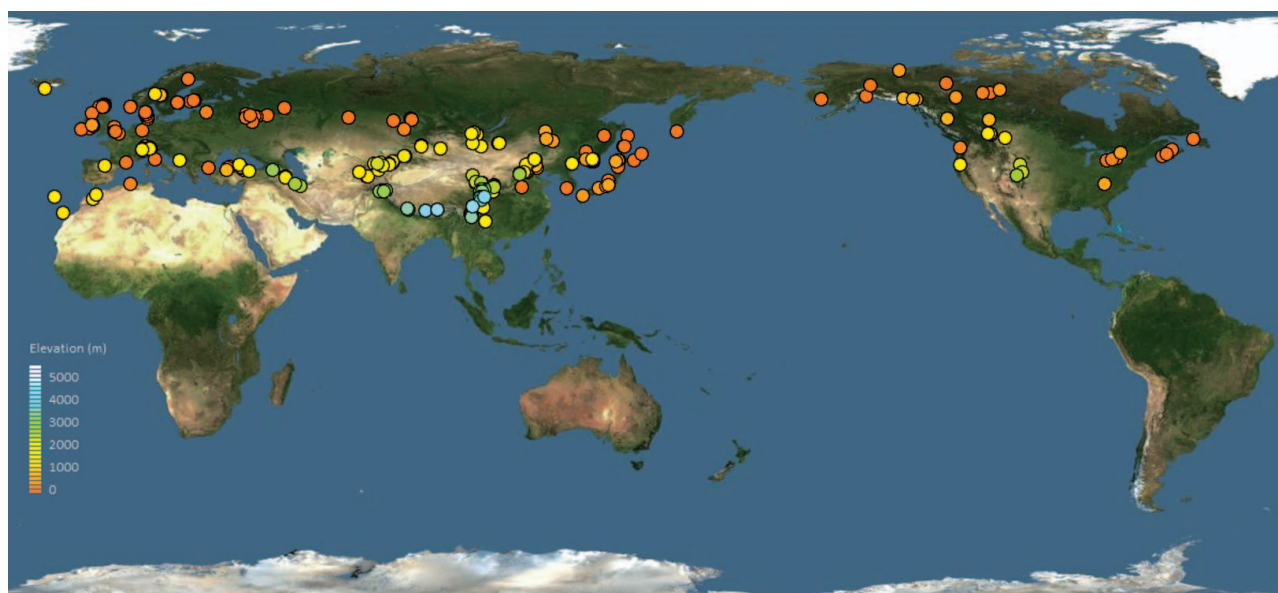


Fig. 1. Global distribution of sites for samples of the subgenus *Bombus s. str.* with accepted COI-barcode sequences (these bumblebees are not indigenous to subSaharan Africa, the Arabian peninsula, lowland India, southeast Asia, Australia or Central and South America). Spots are coloured to show the site elevation (scale in metres at left). Cartesian orthonormal projection, north at the top of the map.

Table 2. Fit of single- and multiple-threshold variants of the GMYC model to lineage-branching patterns.

| Model | T | S_{GMYC} | (S_{GMYC} 95% confidence interval) | L_0 | L_{GMYC} | Likelihood ratio |
|----------|---------|------------|---------------------------------------|---------|------------|----------------------|
| single | −0.0159 | 19 | 4–41 | 1001.41 | 1004.06 | 5.30 ($P = 0.15$) |
| multiple | −0.0159 | 35 | 21–60 | 1001.41 | 1007.91 | 13.01 ($P = 0.07$) |
| | −0.0131 | | | | | |
| | −0.0087 | | | | | |
| | −0.0056 | | | | | |
| | −0.0034 | | | | | |

T , thresholds, the sequence divergence from the branch tips to where the transitions occurred; S_{GMYC} , the number of putative species as the sum of groups and singletons at the thresholds (excluding the 3 outgroups); L_0 , likelihood for the null model that there is a single species; L_{GMYC} , likelihoods for the GMYC models; likelihood ratios evaluated using a chi-square test with 3 degrees of freedom to compare GMYC models and the null model.

reconstruction for the taxon *terrestricoloratus*, see Appendix) are accepted because they lacked indels or in-frame stop codons. They had closely similar GC% (23.3%, SE \pm 0.03) with a strong AT-bias in the third codon position (95.1%, SE \pm 0.05), so that they are unlikely to be from nuclear paralogous copies of the COI gene (‘NUMTs’). The 559 accepted sequences include 130 unique haplotypes with 216 variable nucleotide sites that are potentially informative for differentiating groups.

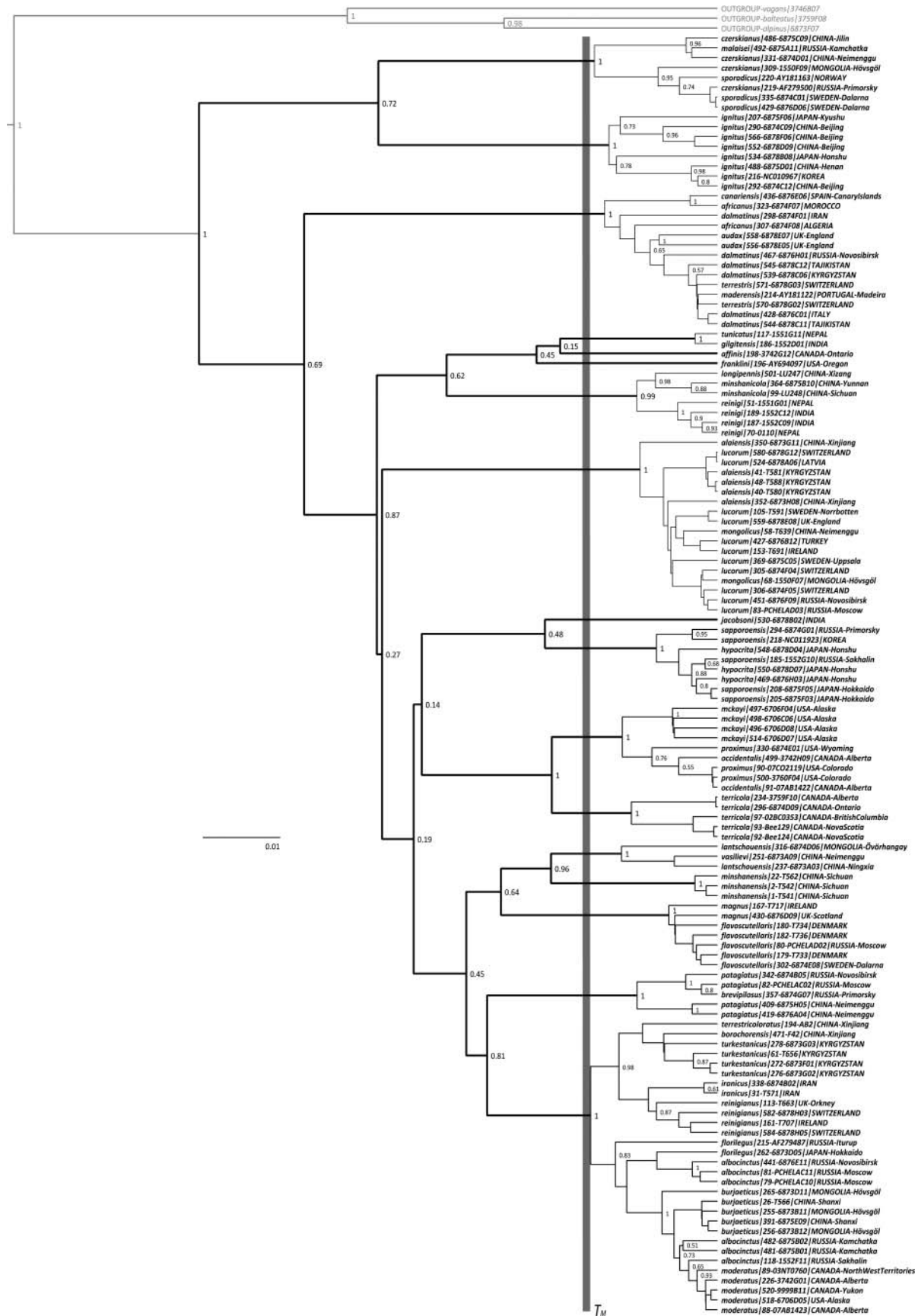
Species recognised

Applying GMYC models to the Bayesian ultrametric tree for the unique haplotypes (Fig. 2) finds the maximum likelihood (Fig. 3) for the transition in branching rate (Fig. 4) between inter- and intraspecific branching patterns. However, neither the single- nor multiple-threshold models shows a significant increase in the log-likelihood scores compared with the (biologically unrealistic) null model of there being just one species in the data (Table 2, Fig. 3; there is no significant difference in the fit between the single- and multiple-threshold models, $\chi^2_{12} = 7.71$, $P = 0.81$). This reflects the relatively uniform branching rate in these data (Fig. 4), which may be part of the reason why species recognition in this group has been problematic. Nonetheless, the fitted thresholds remain the best available guides as to where the largest, biologically significant changes in branching rates occur, even if those changes are not statistically significant.

Of the set of four a priori species, two (*B. affinis*, *B. franklini*) had only a single haplotype in our samples, and therefore could not be used to assess the results. *B. tunicatus* showed two unique haplotypes (one of them representing the taxon *gilgitensis*) and these are recognised as conspecific in both GMYC analyses. Therefore, the relevant difference between the GMYC models lies in the treatment of the haplotypes of *B. ignitus*. The multiple-threshold model splits the seven sampled haplotypes of *B. ignitus* into three entities (prospective ‘species’), whereas the single-threshold model shows a single entity. Examination of the specimens in the three split groups shows no apparent corresponding pattern among them in pile colour pattern, morphology or geographic distribution (more broadly across the tree, the multiple-threshold model generally splits off many entities that similarly show no coherent morphological or geographic patterns). Consequently we have no external evidence to corroborate these splits and so no reason to reject the initial assumption of the conventional single broad species *B. ignitus*. Therefore, by accepting the a priori species to ‘calibrate’ our results, we suggest that the single-threshold model is a more parsimonious fit for the subgenus as a whole.

In addition, we note that a slight adjustment to the GMYC single threshold would avoid geographic morphology-based taxa being divided between these putative species. The two nodes closest to the original threshold (the coalescent points of *B. sporadicus* and *B. cryptarum* in their broad senses) appear to split geographic colour forms

Fig. 2. Bayesian ultrametric tree for longer-sequence exemplars of the 130 unique COI-barcode haplotypes (from the total sample of 559 sequences, frame length 658 nucleotides) of the subgenus *Bombus* s. str. with the single threshold GMYC model applied. This is the consensus of a sample of 9001 trees after 10% burn-in from 40 million generations of the MCMC algorithm. The outgroups (*B. vagans*, *B. alpinus*, *B. balteatus*) were chosen from the results of Cameron *et al.* (2007). Values next to the nodes are Bayesian posterior probabilities for groups (groups with values < 0.9 are considered unreliable, many values < 0.5 have been removed within species). The scale bar represents 0.01 substitutions per nucleotide site. The adjusted single threshold (T_M) from the GMYC model is shown by the vertical grey bar (see Figs 3, 4) and the intersecting lineages are interpreted as subtending separate species. Each unique haplotype is represented by one of the longest exemplary sequences, which has a taxon name (assigned in Fig. 5 from Appendix) and a code that consists of an identifier (B#) from the project database and (after the hyphen) from BOLD (or from another external database, such as GenBank), followed with a reference to its geographic origin.



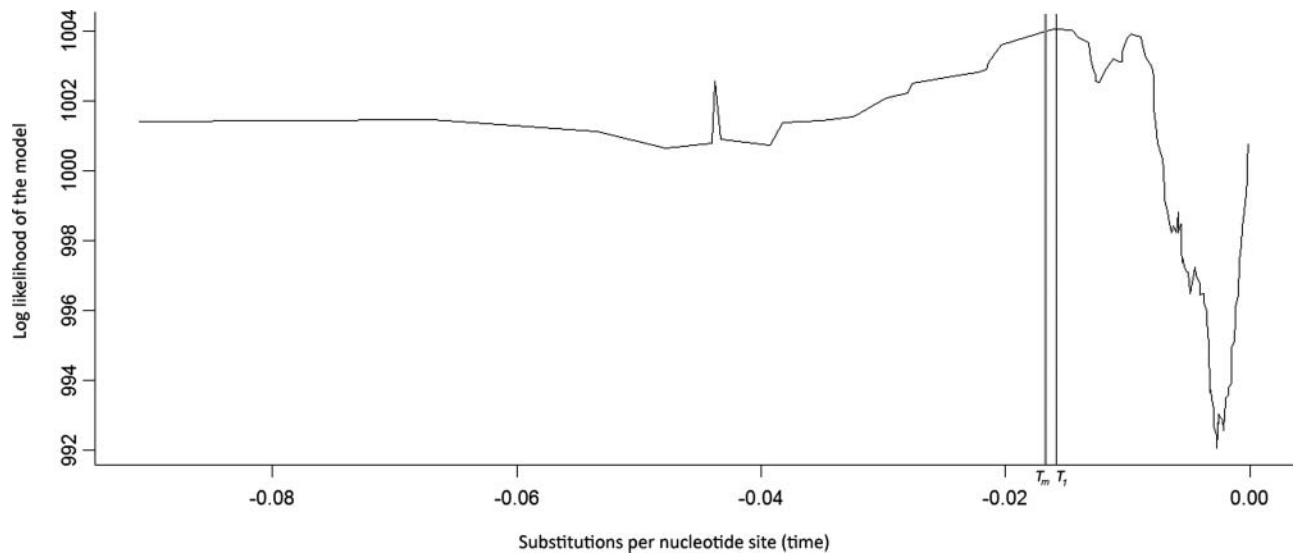


Fig. 3. Plot of (y axis) the log likelihood of the single threshold GMYC model for the Bayesian tree of unique COI-barcode haplotypes against (x axis) substitutions per nucleotide site. T_l , single threshold from the GMYC model at maximum likelihood; T_m , adjusted threshold (see text).

between daughter groups in a way that is inconsistent and so far unsupported by other evidence. There is relatively little COI-barcode divergence in either case between the subgroups compared with between species. Reuniting the parts of these two putative species by adjusting the single threshold from -0.0159 to -0.0166 substitutions per site makes a negligible difference to the log likelihood (Fig. 3) and is an intuitively sensible definition of the point of greatest change in branching rate across the subgenus

Bombus s. str. (Fig. 4). The resulting reduction from 19 to 17 in the number of putative species is small in relation to the 95% confidence limits of 4–41 putative species (Table 2).

After adjusting the GMYC single threshold, all of the species recognized from the phylogenetic tree in Fig. 2 have posterior probabilities for the coalescent groups that are > 0.99 , so the putative species are all reliably monophyletic groups according to this analysis. In total,

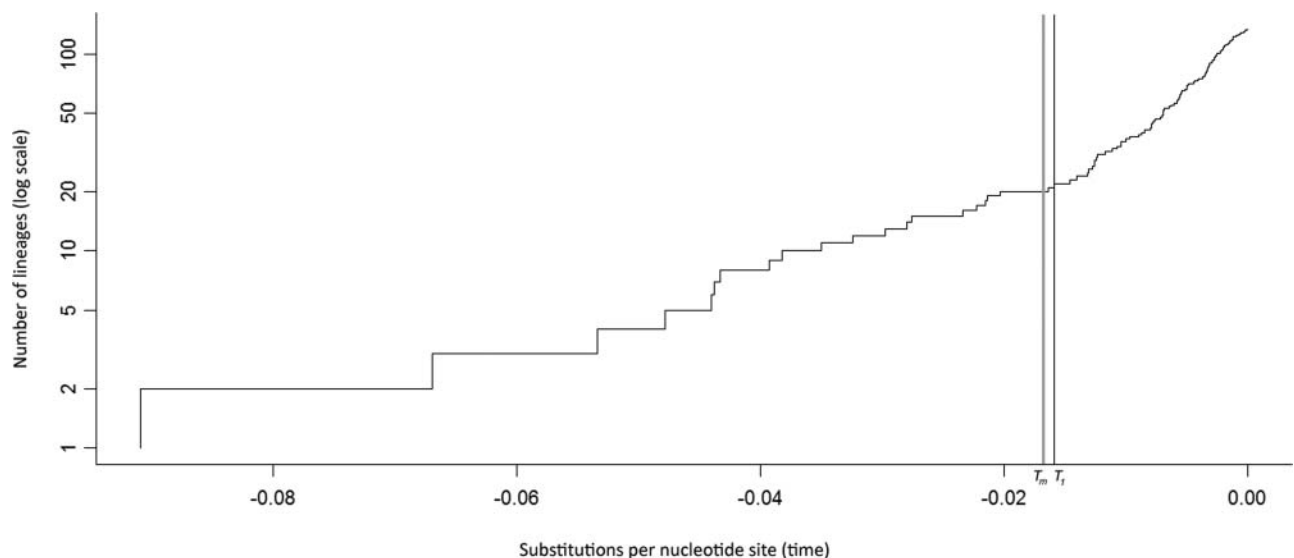


Fig. 4. Plot of (y axis) the number of lineages (N) in the Bayesian tree of unique COI-barcode haplotypes (including the three outgroups) on a log scale against (x axis) substitutions per nucleotide site. T_l , single threshold from the GMYC model at maximum likelihood; T_m , adjusted threshold (see text).

Table 3. Nucleotide changes that in combination are diagnostic for each species from Fig. 2.

| Species | 49: | 235: | 290: | 309: | 322: | 334: | 343: | 359: | 388: | 394: | 400: | 403: | 407: | 409: | 421: | 437: | 484: | 514: | 432: | 619: | 628: |
|--------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| <i>B. sporadicus</i> | A→T | T→C | T→A | C→T | T→A | T→A | T→A | T→C | T→C | T→C | T→C | A→T | A→G | A→T | T→C | T→A | T→C | A→C | T→C | T→A | A→T |
| <i>B. ignitus*</i> | 74: | 79: | 143: | 193: | 220: | 297: | 302: | 308: | 376: | 418: | 529: | 536: | 568: | 610: | | | | | | | |
| | T→C | A→T | T→C | T→C | A→T | G→C | A→T | A→T | T→A | T→C | A→T | T→C | A→T | T→C | | | | | | | |
| <i>B. terrestris</i> | 5: | 10: | 49: | 55: | 88: | 169: | 181: | 197: | 220: | 283: | 337: | 418: | 448: | 542: | 565: | 640: | | | | | |
| | A→T | T→C | A→T | T→A | A→T | A→T | A→T | T→C | A→C | T→C | T→A | T→C | T→C | T→C | A→T | T→C | | | | | |
| <i>B. tunicatus*</i> | 13: | 22: | 130: | 184: | 266: | 290: | 292: | 293: | 304: | 337: | 346: | 347: | 364: | 496: | 526: | 529: | 541: | 562: | 604: | 641: | 646: |
| | T→C | T→C | T→A | A→T | T→C | T→C | A→G | T→C | A→G | T→A | T→A | T→C | T→C | T→C | T→A | A→T | T→C | T→A | C→T | T→C | T→C |
| <i>B. affinis*</i> | 62: | 196: | 265: | 335: | 343: | 370: | 394: | 403: | 461: | 548: | 574: | 634: | 652: | 653: | | | | | | | |
| | A→G | T→C | T→A | G→A | T→C | A→T | T→C | A→G | A→T | T→C | T→A | T→C | T→C | T→C | | | | | | | |
| <i>B. franklini*</i> | 283: | 442: | 526: | 583: | 598: | 601: | | | | | | | | | | | | | | | |
| | T→C | T→C | T→C | T→C | T→C | T→C | | | | | | | | | | | | | | | |
| <i>B. longipennis</i> | 214: | 337: | 634: | | | | | | | | | | | | | | | | | | |
| | A→T | T→A | T→C | | | | | | | | | | | | | | | | | | |
| <i>B. lucorum</i> | 59: | 133: | 302: | | | | | | | | | | | | | | | | | | |
| | T→C | A→T | A→T | | | | | | | | | | | | | | | | | | |
| <i>B. jacobsoni</i> | 265: | 283: | 317: | 340: | 574: | | | | | | | | | | | | | | | | |
| | T→A | T→C | G→A | T→C | T→A | | | | | | | | | | | | | | | | |
| <i>B. hypocrita</i> | 238: | 278: | 303: | 355: | 359: | 454: | 458: | 460: | 533: | 578: | 580: | | | | | | | | | | |
| | T→C | T→C | T→C | T→C | T→C | T→A | T→C | A→T | T→C | T→C | A→T | | | | | | | | | | |
| <i>B. occidentalis</i> | 55: | 109: | 136: | 163: | 212: | 271: | 287: | 289: | 352: | 553: | 562: | | | | | | | | | | |
| | T→A | A→G | T→C | A→T | C→T | T→C | T→C | A→T | T→C | A→G | T→C | | | | | | | | | | |
| <i>B. terricola</i> | 85: | 181: | 302: | 484: | 526: | 583: | 619: | | | | | | | | | | | | | | |
| | T→A | A→G | A→T | T→C | T→C | T→A | T→A | | | | | | | | | | | | | | |
| <i>B. lantschouensis</i> | 172: | 335: | 382: | 557: | 625: | | | | | | | | | | | | | | | | |
| | T→C | G→A | T→C | T→C | A→G | | | | | | | | | | | | | | | | |
| <i>B. minshanensis</i> | 142: | 187: | 430: | 547: | | | | | | | | | | | | | | | | | |
| | T→C | T→C | T→C | T→C | | | | | | | | | | | | | | | | | |
| <i>B. magnus</i> | 10: | 74: | 106: | 212: | 271: | 337: | 412: | 484: | 616: | | | | | | | | | | | | |
| | T→C | T→C | T→C | C→A | T→A | T→A | A→T | T→C | T→C | | | | | | | | | | | | |
| <i>B. patagiatus</i> | 346: | 449: | 479: | 520: | 598: | 625: | 631: | | | | | | | | | | | | | | |
| | T→A | G→A | T→C | T→C | T→C | A→G | A→G | | | | | | | | | | | | | | |
| <i>B. cryptarum</i> | 220: | 287: | 289: | 337: | | | | | | | | | | | | | | | | | |
| | A→C | T→C | A→T | T→A | | | | | | | | | | | | | | | | | |

Numbers show the in-frame nucleotide position, letters show the nucleotides: A, adenine; C, cytosine; G, guanine; T, thymine. Changes shown in bold on grey are non-homoplasious inside or outside species, as unique diagnostics of species within the subgenus *Bombus s. str.*. *Species recognised a priori from distinctive morphology.

Table 4. List of the valid names of the species recognized (shown in bold) and synonyms from studied taxa within the subgenus *Bombus s. str.*

| | |
|--|--|
| <i>Bombus sporadicus</i> Nylander, 1848 <i>czerskianus</i> Vogt, 1911 <i>malaisei</i> Bischoff, 1930 | <i>Bombus jacobsoni</i> Skorikov, 1912 |
| <i>Bombus ignitus</i> Smith, 1869 | <i>Bombus hypocrita</i> Pérez, 1905 <i>sapporoensis</i> Cockerell, 1911 |
| <i>Bombus terrestris</i> (Linnaeus, 1758) <i>audax</i> (Harris, 1776) <i>xanthopus</i> Kriechbaumer, 1870 <i>dalmatinus</i> Dalla Torre, 1882 <i>canariensis</i> Pérez, 1895 <i>terrestriformis</i> Vogt, 1911 <i>lusitanicus</i> Krüger, 1956 <i>africanus</i> Vogt in Krüger, 1956 <i>maderensis</i> Erlandsson, 1979 | <i>Bombus occidentalis</i> Greene, 1858 <i>proximus</i> Cresson, 1863 <i>mckayi</i> Ashmead, 1902 |
| <i>Bombus tunicatus</i> Smith, 1852 <i>gilgitensis</i> Cockerell, 1905 | <i>Bombus terricola</i> Kirby, 1837 |
| <i>Bombus affinis</i> Cresson, 1863 | <i>Bombus lantschouensis</i> Vogt, 1908, STAT. NOV. <i>vasilievi</i> Skorikov, 1913 <i>beickianus</i> Bischoff, 1936 <i>pseudosporadicus</i> Bischoff, 1936 |
| <i>Bombus franklini</i> (Frison, 1921) | <i>Bombus minshanensis</i> Bischoff, 1936, STAT. NOV. |
| <i>Bombus longipennis</i> Friese, 1918 <i>minshanicola</i> Bischoff, 1936 <i>reinigi</i> Tkalcu, 1974, SYN. N. | <i>Bombus magnus</i> Vogt, 1911 <i>flavoscutellaris</i> G. & W. Trautmann, 1915 <i>luteostriatus</i> Krüger, 1954 |
| <i>Bombus lucorum</i> (Linnaeus, 1761) <i>?alaiensis</i> Reinig, 1930 <i>?mongolicus</i> Krüger, 1954 | <i>Bombus patagiatus</i> Nylander, 1848 <i>ganjsuensis</i> Skorikov, 1913, SYN. N. <i>brevipilosus</i> Bischoff, 1936 |
| | <i>Bombus cryptarum</i> (Fabricius, 1775) <i>albocinctus</i> Smith, 1854 <i>moderatus</i> Cresson, 1863 <i>terrestricoloratus</i> Krüger, 1951 <i>iranicus</i> Krüger, 1954 <i>?borochorensis</i> Krüger, 1954 <i>?turkestanicus</i> Krüger, 1954 <i>?burjaeticus</i> Krüger, 1954 <i>florilegus</i> Panfilov, 1956 <i>reinigianus</i> Rasmont, 1984 <i>armeniensis</i> Rasmont, 1984 |

Species are recognised using the GMYC model in Fig. 2 and named from the oldest available names associated with the proxy types in Fig. 5 (see Appendix). Synonyms (shown in reduced font, some of which may be regarded as subspecies, see text) are included only where they relate to taxa represented in Fig. 5. Question marks show names for which the association between the primary type and the proxy type is less reliable (see the Appendix).

Note added in proof: after submission of the manuscript, two different haplotype sequences were obtained from specimens from Lama, Corsica, for *Bombus terrestris xanthopus* Kriechbaumer. This taxon is endemic to the islands of Corsica, Capraia and Elba, and has a strongly divergent colour pattern from continental *B. terrestris*, characterised by an orange tail and no yellow bands (Rasmont *et al.*, 2008). Re-running the BEAST analysis shows that these haplotypes form a strongly supported group within *B. terrestris*, as a sister group to the haplotype group that includes sequences 545 539 571 214 428 544 570 (see Fig. 2).

483 nucleotide changes were observed across the tree. Each of the putative species can be diagnosed by a unique combination of shared nucleotide changes from its basal coalescent branch (Table 3).

Species' names

We relate the species recognized using the GMYC adjusted single-threshold model (Fig. 2) via the proxy type se-

quences (Fig. 5, Appendix) to their oldest available names (Table 4). Aside from the a priori species (*B. affinis*, *B. franklini*, *B. ignitus*, *B. sporadicus* and *B. tunicatus*), the putative species from our analysis all correspond to modified concepts of previously named taxa, for which the oldest available names are *B. cryptarum*, *B. hypocrita*, *B. jacobsoni*, *B. lantschouensis*, *B. longipennis*, *B. lucorum*, *B. magnus*, *B. minshanensis*, *B. occidentalis*, *B. patagiatus*, *B. terrestris* and *B. terricola*. Among these, *B. lantschouensis*

and *B. minshanensis* are recognised for the first time as having the status of separate species. In contrast, some taxa that have previously been regarded as separate species are instead interpreted here as parts of broader species, including the taxa *audax*, *burjaeticus*, *canariensis*, *gilgitensis*, *maderensis*, *moderatus*, *sapporoensis* and *vasilievi*.

some for the first time (Table 4). Some or all of these taxa no longer recognised as separate species could still be regarded as having subspecific rank, if subspecies were considered useful for labelling parts of species, particularly where there appears to be evidence of monophyletic groups from the COI barcodes.

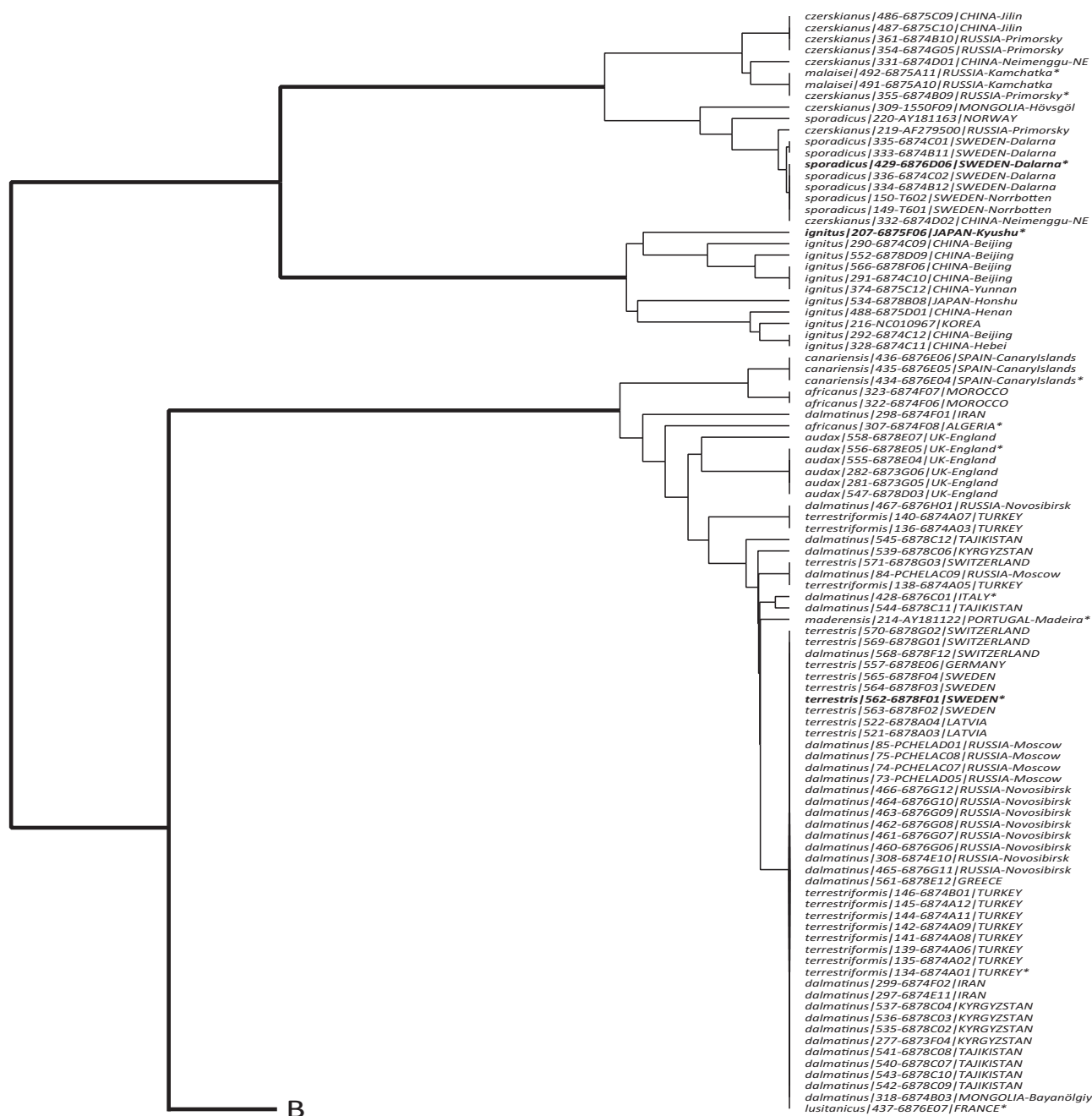


Fig. 5. Estimate of phylogeny for unique haplotypes from Fig. 2 with accepted sequences interpolated back in. Because some sequences differ in length, shorter sequences may match more than one longer unique haplotype and these ambiguous shorter sequences are not shown. The codes following the taxon names follow the format used in Fig. 2. Lineages subtending species are shown with thick branches. Asterisks mark samples used as informal proxies for the type specimens of each of the principal taxon names (see Appendix). The proxy sample for the type specimen for the oldest available name (the valid name) for each species (from Fig. 2) is marked in bold.

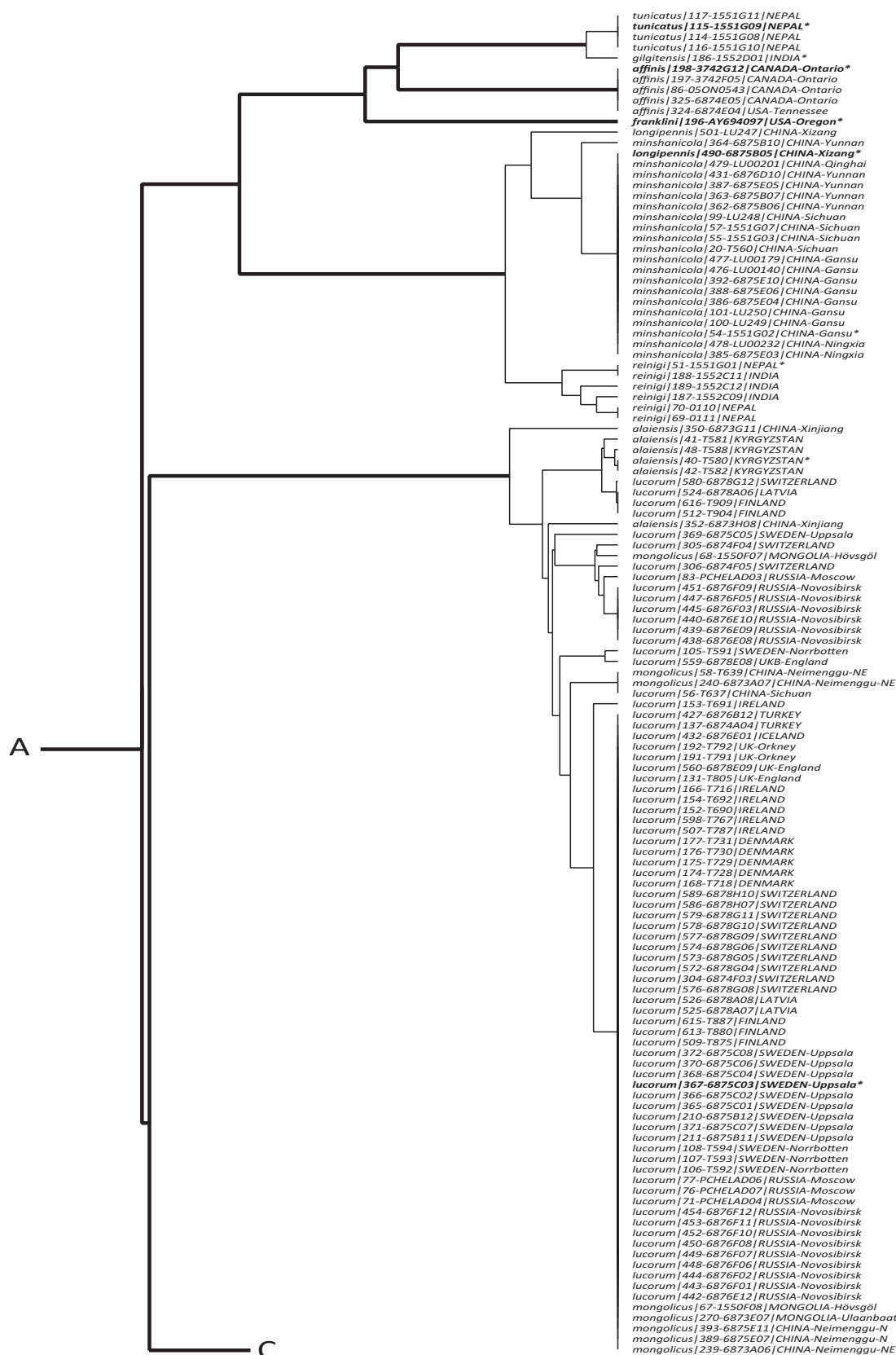


Fig. 5. (Continued)

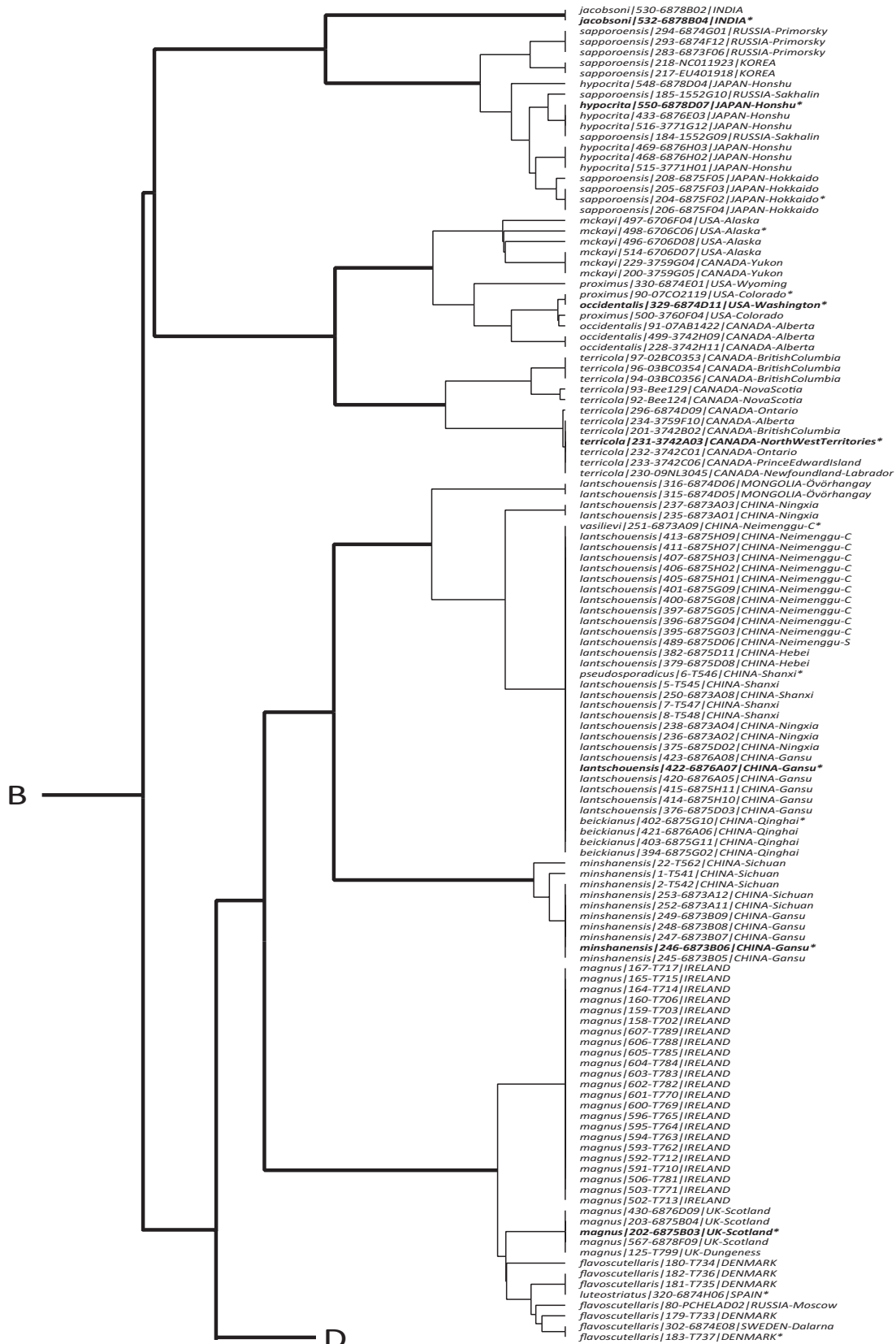


Fig. 5. (Continued)

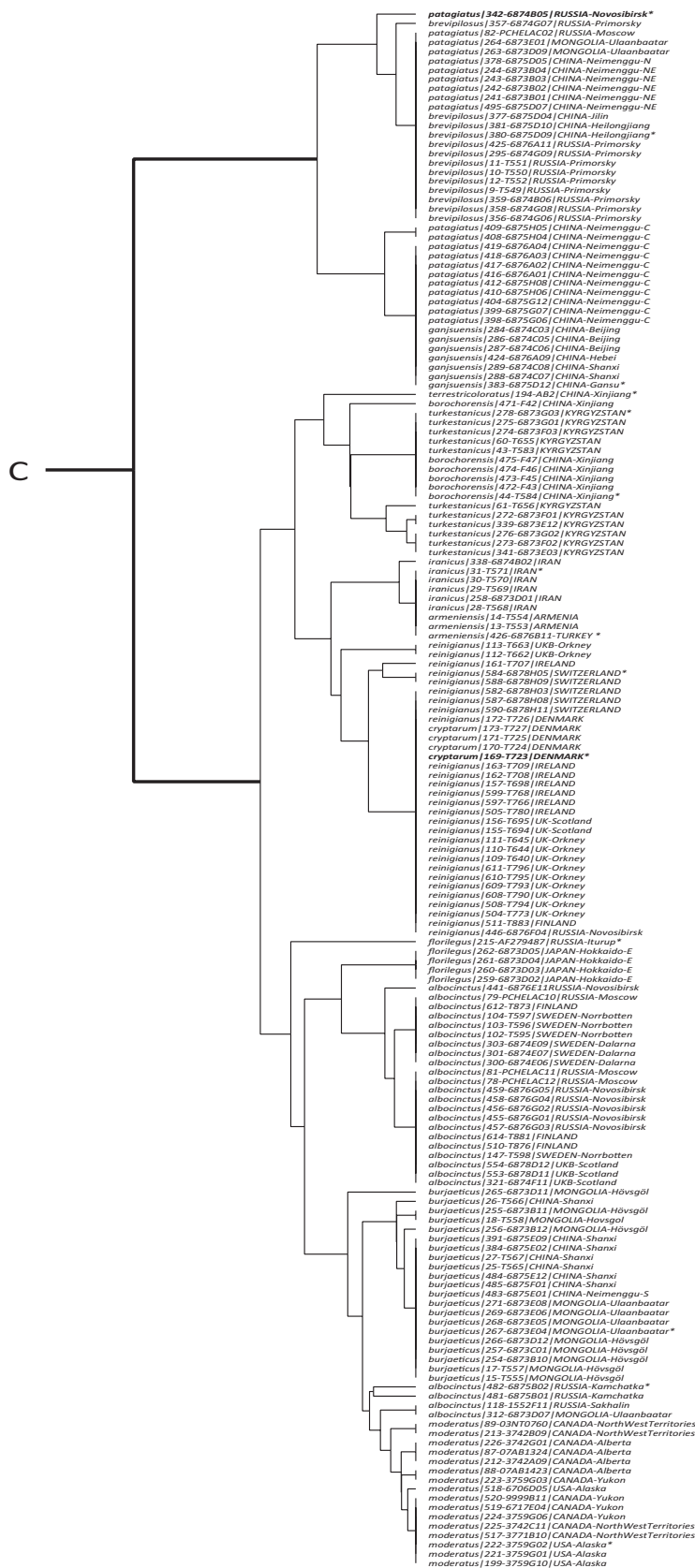


Fig. 5. (Continued)

Species' geographic distributions

Recognising putative species among all accepted COI-barcode sequences (Fig. 5) allows us to map the broader geographic distributions of these species (Figs 6–22). The maps include grey spots to show the sites from which specimens of all species with accepted sequences were recorded, whereas the black spots show records for individual species using specimens identified from their sequences. Absence of a record for a particular species (i.e. a grey spot) cannot be interpreted as indicating the absence of the species. The grey spots are useful for comparing a species' presence records (black spots) with the geographic distribution of sampling effort in this study.

Some of the more important geographic results are that: (1) *B. terrestris* (Fig. 8) is recorded further west in the Azores and further east in Mongolia; (2) *B. lucorum* (Fig. 13) is widespread in the Palaearctic including Central Asia and with a record from Iceland and a single specimen from the Oriental region, in Sichuan; (3) *B. jacobsoni* (Fig. 14) has a small known range, endemic to Kashmir; (4) *B. hypocrita* (Fig. 15) is not recorded from China; (5) *B. occidentalis* is divided into northern and southern populations (Fig. 16) with distinct haplotype groups (Fig. 2); (6) *B. lantschouensis* (Fig. 18) is recorded from both north and south of the Mongolian arid zone; (7) *B. minshanensis* (Fig. 19) is endemic to China; (8) *B. magnus* (Fig. 20) is confirmed as unknown from east of the Urals (Levchenko, 2010); (9) *B. patagiatus* (Fig. 21) is recorded from both north and south of the Mongolian arid zone, including a southern population that was previously misidentified; (10) *B. cryptarum* (Fig. 22) is the most widespread species of the subgenus and extends into Central Asia, north China, the Kuril Islands and north-western North America.

Discussion

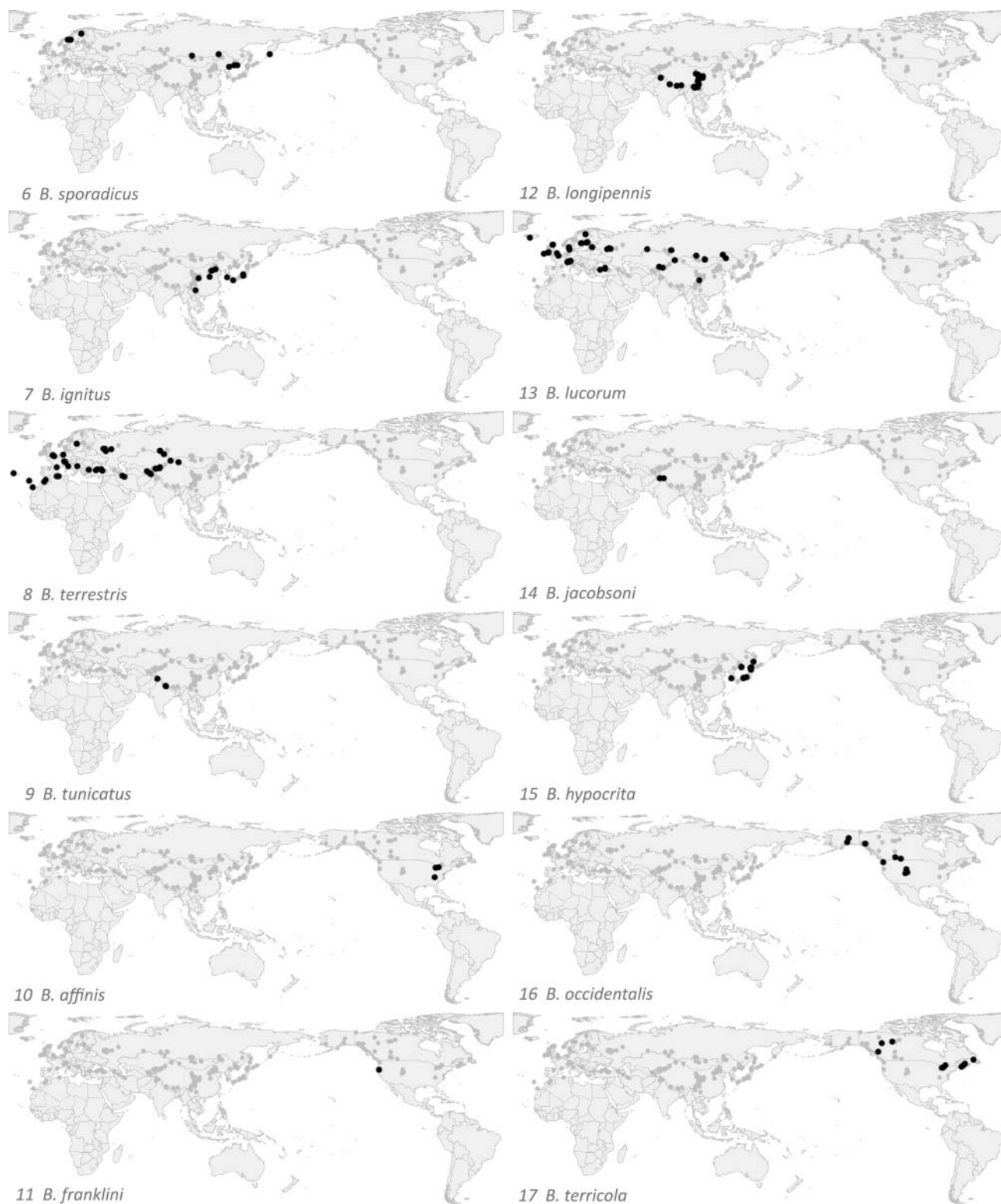
Caveats

Potential sources of larger error in this study include the following. The arctic part of the Palaearctic region may be under-represented in our sampling (Fig. 1). Museum specimens are likely to suffer from damaged DNA in some older specimens. Some previously published sequences have been identified as damaged (cf. Bertsch, 2009) and we have removed these from our analyses. Extraction, amplification, and sequencing were done in three separate laboratories, so it is possible that each laboratory has its own biases. However, no segregation of groups by laboratory was detected in the results. Non-target DNA can be amplified by mistake, such as from *Wolbachia* infections (Werren, 1997; Ballard & Whitlock, 2004). This was tested for and rejected (although the presence of *Wolbachia* elsewhere within specimens or its effects were not tested, Gerth *et al.*, 2011). The evolutionary model used to build the tree is inevitably an approximation. However, as new sam-

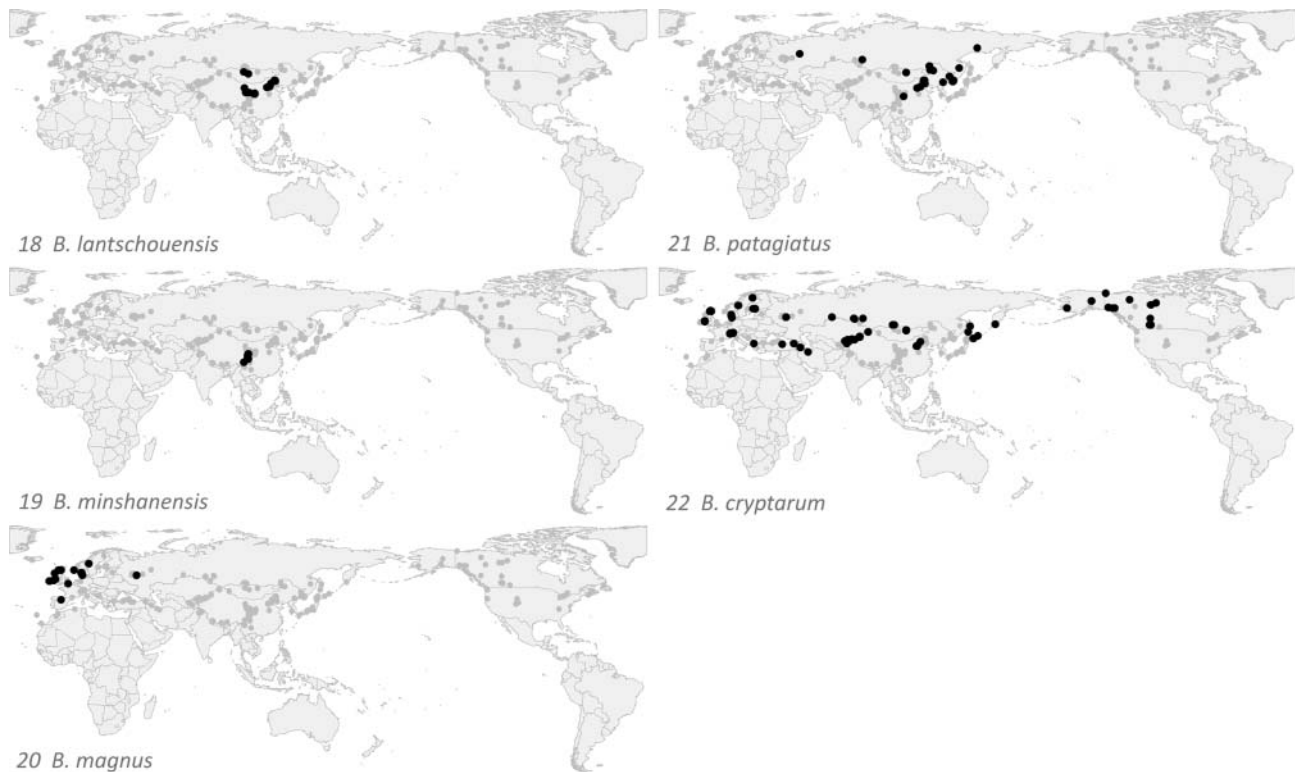
ples were acquired and added to the analyses, the results did not show substantial instability in the topology of the tree. Phylogenetic inferences for closely related taxa derived from DNA-sequence data are more sensitive to the number of individuals sampled than to the number of genes sampled (Maddison & Knowles, 2006). With 559 sequences accepted, this study includes the largest number of individuals sampled so far for COI barcodes within the subgenus *Bombus s. str.*, representing most of the global distributions for all of the major taxa insofar as they are known (Fig. 1). However, there are long branches near the base of the tree, where extant populations have greater distances between them. To improve the models in this region, we have added more outgroups, although we expect inferences of these relationships (e.g. between *B. sporadicus* and *B. ignitus*) to be less reliable. Patterns of evolution for mitochondrial genes might not correspond precisely with those of their owner organisms, either because of incomplete lineage sorting of ancestral polymorphisms (Edwards & Beerli, 2000; Maddison & Knowles, 2006); or because copies become transferred to the nucleus as mitochondrial pseudogenes and show altered patterns of evolution ('NUMTs': Song *et al.*, 2008); or because of introgression between species (Ballard & Whitlock, 2004; Monaghan *et al.*, 2005), such as via leakage of paternal mitochondrial DNA (Magnacca & Brown, 2010). NUMTs were rejected because of an unchanged AT frequency and no evidence of introgression was found from obviously misplaced groups in the tree. Even mitochondrial DNA might not always change fast enough to track recent speciation, so sister species could retain identical sequences (Kaila & Stahls, 2006; Kuhlmann *et al.*, 2007; Skevington *et al.*, 2007). We have no evidence of this from pronounced morphological heterogeneity within terminal groups. Thus, despite these and other potential pitfalls of using COI barcodes (Rubinoff *et al.*, 2006), no problems have manifested themselves to an obvious or serious extent. So far, COI barcodes appear to have provided cost-effective data to supplement the sparse morphological information, to enable us to diagnose otherwise cryptic putative species. But our interpretation is necessarily provisional until it can be assessed with independent data from appropriate independently inherited genes.

Phylogeny and species groups

Our inference of phylogeny for species of the subgenus *Bombus s. str.* (Fig. 2) shows low posterior probabilities for many higher groups, so these groups are unreliable. Nonetheless, the topology is similar to the results of the most recent previous inference of phylogeny for the subgenus by Bertsch (2010: his fig. 2). Bertsch's study used longer sequences of COI, but otherwise covered less of the total variation, with 13% as many sequences as in our analysis, and not including several of the species recognised here (*B. lantschouensis*, *B. minshanensis*, *B. jacobsoni*) or



Figs 6–17. Global distribution of material with accepted COI-barcode sequences for each species in turn (black spots) superimposed on combined barcoded records for all species (grey spots). Cartesian orthonormal projection (excluding Antarctica), north at the top of the map.



Figs 18–22. Global distribution of material with accepted COI-barcode sequences for each species in turn (black spots) superimposed on combined barcoded records for all species (grey spots). Cartesian orthonormal projection (excluding Antarctica), north at the top of the map.

many of the regional parts of larger populations recognised previously as divergent (his *B. minshanicola* is part of our *B. longipennis*). Just as in our results, his fig. 2 showed basal relationships for *B. sporadicus*, *B. ignitus* and *B. terrestris*. It then showed a similar pattern of relatively weak support for the relationships of *B. hypocrita* and *B. lucorum*. An earlier study by Cameron *et al.* (2007: their fig. 1) was based on five other genes, including four nuclear genes (these are more independent of COI, although often slower evolving and less informative), but from a total of just 13 specimens. The topology of their tree is broadly similar in the branching order of species to Fig. 2 (their ‘lucorum (China)’ is *B. longipennis* and their ‘patagiatus’ is *B. minshanensis*). Therefore our results, based only on short COI barcodes, have recovered similar inferences of relationship to earlier results from longer COI sequences and from more genes. This increases our confidence in our results, which cover much larger samples of the variation among individuals worldwide.

Recognising species

Using easily available COI-barcode data, the GMYC models provide accountable interim results for the difficult species, despite the relatively homogeneous branching rates for this group. Our approach is distinct from the DNA Bar-

coding Enterprise (*sensu* Ebach & Carvalho, 2010) and we aim to follow it up with studies integrating other character systems (Will *et al.*, 2005).

A number of conditions apply. For example, the GMYC models assume reciprocal monophyly among species. Reciprocal monophyly is rejected by some as a necessary condition of species (Knowles & Carstens, 2007), whereas pursuit of monophyletic taxa defines ‘best practice’ in systematics for others (Ebach & Carvalho, 2010). Accepting some of the taxa that have been accepted previously as species within the subgenus *Bombus s. str.* (e.g. the taxa *florigilus*, *moderatus*) would require the acceptance of other paraphyletic species according to our results. However, examination of the results (Figs 2, 5) shows that these paraphyletic taxa also show weak differentiation in COI barcodes. As far as we can tell from this evidence, there is little justification for accepting them as species other than their obvious but minor differences in either semi-melanic pile colour pattern or disjunct geographic distribution. These characteristics have not been accepted widely as sufficient among regional colour-pattern variations of *B. terrestris* in Europe (Estoup *et al.*, 1996; Rasmont *et al.*, 2008).

Applying a single threshold across the tree to identify species does not assume that all of the species have the same age of their most recent common ancestor, but rather that the most recent speciation event occurred before the

oldest detected within-species coalescence (Pons *et al.*, 2006). This makes the single-threshold model conservative and unable to detect any more-recently diverged species (Papadopoulou *et al.*, 2008). From an examination of Fig. 2, two cases are particularly likely to come under consideration again in the future, involving the putative species *B. cryptarum* and *B. occidentalis* (see below). Less likely cases occur within *B. hypocrita*, *B. longipennis*, *B. patagiatus*, *B. terrestris* and *B. terricola*.

Our GMYC results placed a single threshold initially at a (GTR+I+G) divergence of 1.59% (Table 2), although our adjusted threshold increased this to 1.66%. In comparison, Monaghan *et al.* (2009) derived similar single GMYC thresholds for four insect groups that ranged between 1–2% (GTR+I+G) divergence, although for dung beetles the threshold was higher at 5%.

The GMYC approach of employing a threshold to distinguish between inter- and intraspecific branching patterns is related to the idea of a 'gap' between inter- and intraspecific levels of COI-barcode divergence discussed in many barcoding studies. Famously, Hebert *et al.* (2004b) described this gap from birds and proposed using a threshold of 2.7% (K2P) divergence as appropriate for recognising species. Subsequent assessments have shown that instead of a gap there is usually an extensive overlap between levels of inter- and intraspecific divergence (Meier *et al.*, 2006). In addition, Meyer and Paulay (2005) found the upper 99% confidence limits for all intraspecific (K2P) divergences to vary substantially, at 2.85% for cowries, 1.38% for limpets and 1.12% for turbinids. From our GMYC results, the largest intraspecific (K2P) divergences within our putative species also range broadly, from 0.31–3.67% (Table 5).

Among 559 sequences we found only 130 unique haplotypes. Although our sampling effort (Fig. 1) was designed to cover the breadth of the known geographic ranges of the species, we may still be far from sampling all of the haplotype variation within those ranges. Future sampling is expected to discover more haplotypes, which should fill in more branching events within species. This is likely to strengthen the GMYC model threshold statistics.

The taxonomic problem with the subgenus *Bombus s. str.* has been that while a few species are distinctive and agreed, most taxa have been of uncertain status, with only weak morphological characters from which to study them. Our estimate of the phylogenetic tree (Fig. 2) shows a strongly asymmetric branching pattern, which implies much longer branches to the extant 'basal' species. Unsurprisingly then, the four species considered a priori morphologically distinctive are clustered in the basal part of the tree, which also has the species with the largest numbers of diagnostic nucleotide changes (upper part of Table 3) and the largest interspecific divergences (upper part of Table 5).

Taxonomic and nomenclatural consequences

Only by assessing the full range of variation among all of the major taxa within a group across their entire global distributions is it possible to model patterns of character change reliably in order to derive the best estimates of relationships. Our results (Fig. 5, Table 3) support recognising

Table 5. Divergence in COI-barcode sequences among and within species from Fig. 2.

| Species | Number of unique haplotypes | Number of (unique) diagnostic polymorphisms | Minimum interspecific sequence divergence (%) | Maximum intraspecific sequence divergence (%) | Mean intraspecific sequence divergence (%) |
|--------------------------|-----------------------------|---|---|---|--|
| <i>B. sporadicus</i> | 8 | 21 (9) | 5.51 | 2.31 | 0.89 |
| <i>B. ignitus</i> * | 8 | 14 (4) | 6.07 | 1.86 | 0.79 |
| <i>B. terrestris</i> | 14 | 16 (6) | 3.77 | 2.21 | 0.73 |
| <i>B. tunicatus</i> * | 2 | 21 (10) | 5.09 | 0.46 | 0.46 |
| <i>B. affinis</i> * | 1 | 14 (7) | 3.84 | — | — |
| <i>B. franklini</i> * | 1 | 6 (2) | 3.04 | — | — |
| <i>B. longipennis</i> | 7 | 3 (0) | 1.86 | 3.67 | 1.72 |
| <i>B. lucorum</i> | 18 | 3 (1) | 1.86 | 1.25 | 0.46 |
| <i>B. jacobsoni</i> | 1 | 5 (0) | 3.49 | — | — |
| <i>B. hypocrita</i> | 8 | 11 (6) | 3.46 | 1.24 | 0.59 |
| <i>B. occidentalis</i> | 9 | 11 (5) | 3.53 | 3.03 | 1.33 |
| <i>B. terricola</i> | 5 | 7 (2) | 2.65 | 0.61 | 0.29 |
| <i>B. lantschouensis</i> | 3 | 5 (3) | 1.57 | 0.33 | 0.21 |
| <i>B. minshanensis</i> | 3 | 4 (3) | 1.57 | 0.31 | 0.26 |
| <i>B. magnus</i> | 7 | 9 (4) | 2.85 | 0.77 | 0.40 |
| <i>B. patagiatus</i> | 5 | 7 (2) | 1.86 | 2.36 | 1.38 |
| <i>B. cryptarum</i> | 30 | 4 (0) | 2.52 | 3.49 | 1.39 |

Sequence divergences are calculated using the Kimura two-parameter model (Kimura, 1980). *Species recognised a priori from distinctive morphology.

relationships for several taxa that require nomenclatural changes.

B. sporadicus (Fig. 6) has many diagnostic nucleotide changes, but we find no evidence from COI barcodes to support the described eastern and western subspecies (cf. Tkalcu, 1967).

B. terrestris (Fig. 8) is strongly supported as a distinctive species. The taxa *canariensis* and *maderensis* have sometimes been regarded as separate species (Erlandsson, 1979; Rasmont, 1984, but not by Estoup *et al.*, 1996; Rasmont *et al.*, 2008; Bertsch, 2010), but this is not supported here. The taxon *canariensis* shares one diagnostic change (position 250) with *africanus* from Morocco, and has one other unique diagnostic change of its own (position 122). The Turkish taxon *terrestriformis* is indistinguishable from the southern European *dalmatinus* in their COI barcodes. *B. terrestris* has been introduced beyond the indigenous range mapped here, into New Zealand, Tasmania, South America and Japan (Arretz & Macfarlane, 1982; Macfarlane & Gurr, 1995; Stout & Goulson, 2000; Matsumura *et al.*, 2004; Torretta *et al.*, 2006; Schmid-Hempel *et al.*, 2007; Inoue *et al.*, 2008). Because this species has proved to be invasive and to have deleterious effects on native species (*op. cit.*), it should not be introduced elsewhere until proven safe, and especially not beyond its range limit to the east and south of the Mongolian arid zone.

B. longipennis (Fig. 12) has no uniquely diagnostic changes, although there are uniquely diagnostic changes for taxa within the species: six for *longipennis s. str.* (southern Tibet and possibly Sikkim) and three for *reinigi* (western Himalaya). With further evidence these taxa might be split as separate species. The lack of unique diagnostic changes for *B. longipennis s. l.* could reflect the way the distribution of this species may have become rapidly fragmented by climatic fluctuations within some of the highest mountain ranges in the world. Using other genes, this species has previously been recognised as separate from both *B. lucorum* and *B. cryptarum* under the name ‘*lucorum* (China)’ by Cameron *et al.* (2007). Bertsch (2010) and Bertsch & Schweer (2011) also agreed that it is separate, but had then used Bischoff’s more recent name *B. minshanicola* for the species. Previously, from morphological evidence, Tkalcu (1974a) had not only recognised that the species is separate from *B. lucorum*, but had also already synonymised the taxon *minshanicola* with the species *B. longipennis* (noted also by Rasmont *et al.*, 1986). In contrast, Bertsch *et al.* (2010a) had concluded from a single ‘degraded’ COI sequence that the taxon *reinigi* is a separate species and part of the ‘*cryptarum*-complex taxa’.

B. lucorum (Fig. 13) has only a single unique diagnostic change, but is broadly distributed with many unique nucleotide changes within it. If our proxy types are correctly identified (see Appendix), it includes the taxon *alaiensis* in the mountains of Central Asia and the taxon *mongolicus* Krüger (a junior homonym of *mongolicus* Friese, 1916) to

the east, in Mongolia and the region to the east of Lake Baikal. From morphology, both taxa had previously been rejected as parts of *B. lucorum* (Rasmont *et al.*, 1986).

B. jacobsoni (Fig. 14) has no known uniquely diagnostic changes. The three available sequences (from two recent individuals) are short (426 nucleotides), but identical. This species appears to rival *B. franklini* (Fig. 11) as one of the more narrowly distributed bumblebee species worldwide (Williams, 1998). Its known distribution is apparently less than 260 km across within Kashmir, where it seems to be rare and easily confused with the far more abundant *B. longipennis* (Williams, 1991). In contrast, Bertsch *et al.* (2010a) concluded that the taxon *jacobsoni* is part of *B. cryptarum*, although they used a single ‘degraded’ COI sequence from an older specimen, collected in 1929 (ZMA).

B. hypocrita (Fig. 15) has unique diagnostic changes. We find no clear evidence from COI barcodes to support separate groups for a single northern, more extensively pale subspecies (*sapporoensis*) and a southern, darker subspecies (*hypocrita s. str.*, Sakagami & Ishikawa, 1972). In contrast, there is weak support (but no unique diagnostic changes) for divergence between mainland and island populations.

B. occidentalis (Fig. 16) is supported by several unique diagnostic changes as a separate species. In revising American bumblebees, Franklin (1913: 239–240) was unsure that it is separate and wrote that he could accept that it is part of *B. terricola*, but then listed them separately. This separation has been widely followed (recently: Thorp & Shepherd, 2005; Evans *et al.*, 2008, but see Milliron, 1971), but without presenting further evidence other than that they co-occur in Alberta (Hobbs, 1968, who was not explicit as to how the taxa were distinguished there). There has been increasing evidence (although from small samples) to support splitting them as two species (Cameron *et al.*, 2007; Bertsch *et al.*, 2010b), which is further supported by our GMYC model results. In addition, there is support from single unique diagnostic changes in each case for two distinct and apparently disjunct groups within *B. occidentalis*: a northern (Alaska to northern British Columbia) group of bees with longer pile (the oldest available name for this northern subspecies is *mckayi*, described previously as a separate species) and a southern (southern British Columbia to the western USA excluding Alaska) group with shorter pile (subspecies *occidentalis s. str.*, which includes the taxon *proximus*, also described previously as a separate species), which are not known to occur together.

B. terricola (Fig. 17) is supported by several diagnostic changes as a separate species. In western North America its distribution appears to extend between the two disjunct populations of its sister-species *B. occidentalis* (Fig. 16).

B. lantschouensis and *B. minshanensis* (Figs 18, 19) are recognised as separate species for the first time and are each supported with three uniquely diagnostic changes. These bees from North China were regarded previously as

parts of the more northern species *B. patagiatus* (Krüger, 1956; Tkalcu, 1967; Rasmont, 1984; Yao & Wang, 2005; Williams *et al.*, 2009; An *et al.*, 2010). In Neimenggu (Inner Mongolia) and Heilongjiang, the taxa *vasilievi* (described previously as a separate species) and *lantschouensis* are indistinguishable from one another in COI barcodes, as parts of the species *B. lantschouensis*.

B. magnus (Fig. 20) has several unique diagnostic changes. The Irish samples are particularly distinct, as reported previously by Carolan *et al.* (2012), although with larger samples this is now supported by only one unique diagnostic change.

B. patagiatus (Fig. 21) has two unique diagnostic changes, but includes a southern population with five unique diagnostic changes. These distinctive southern bumblebees from North China were previously widely regarded as part of the species *B. hypocrita* (Bischoff, 1936; Krüger, 1958; Tkalcu, 1962; Sakagami & Ishikawa, 1969; Ito & Sakagami, 1980; Williams, 1991; Yao & Wang, 2005; An *et al.*, 2010) but are interpreted here as a semi-melanic part of *B. patagiatus* (oldest available name *gansjuensis*). In 2010, field work was targeted on the arid region of Neimenggu (Inner Mongolia) that has few bumblebees, between the pale Russian population to the north and the dark North Chinese population to the south. Individuals collected from the top of one isolated patch of mesic habitat on a mountain (in Huanggangliang) in this otherwise arid region are intermediate in character. They have the COI haplotype and narrow pale bands of the southern *gansjuensis* (which usually has an orange tail) but the white tails of the northern *patagiatus s. str.* (which has broad pale bands). The former confusion between *B. patagiatus* and *B. hypocrita* is important because the dark Chinese *B. patagiatus gansjuensis* has been found to be a good pollinator in glasshouses (under the name *B. hypocrita*: An *et al.*, 2010; Chen *et al.*, 2011; Zhao *et al.*, 2011), as are the true *B. hypocrita* in Japan (Asada & Ono, 1996). It would be unwise to allow this misidentification to encourage the introduction of true Japanese *B. hypocrita* into China (or vice versa) through taxonomic confusion, because this might lead to the loss of one or other indigenous population (cf. Matsumura *et al.*, 2004; Inoue *et al.*, 2008). The combination of these name changes arising from our understanding of the species *B. patagiatus*, *B. lantschouensis* and *B. minshanensis* within North China is unfortunate: it results in the usage of the name *patagiatus* being transferred from one species to another, quite different, species within this region (cf. Tkalcu, 1967). However, this is a biological issue, not a purely nomenclatural issue, so it is not amenable to a simple nomenclatural solution (both the names *B. hypocrita* and *B. patagiatus* have to be retained in their familiar senses for these species elsewhere in their distribution ranges).

B. cryptarum (Fig. 22) has no known uniquely diagnostic changes. It is the most broadly distributed species of the subgenus *Bombus s. str.* in our results and includes

more geographically structured subgroups with diagnostic changes than the other species. There are two principal subgroups, each with a single diagnostic change (and no known diagnostic morphological characters), that are both widespread and might be regarded as subspecies: one more southern (Ireland, Britain, through central Europe, Turkey and Iran to Central Asia; oldest available name *cryptarum s. str.*, including the southern European taxon *reinigianus*); and one more northern (Scotland, Scandinavia, northern Russia, Mongolia and China, to western North America; oldest available name *albocinctus*, although northern European individuals also resemble the taxon *reinigianus* in pile colour pattern). Both subgroups occur in Scotland. They correspond to the two haplotypes reported by Carolan *et al.* (2012) from (1) Denmark, Scotland and Ireland on the one hand; and from (2) Finland on the other (although with larger samples, there are now fewer unique diagnostic changes in our results). From morphology, the taxon *albocinctus* had been interpreted instead as part of *B. lucorum* (Tkalcu, 1974c; Davydova, 2001). In the mountains of Western Asia, the taxa *iranicus* and *armeniensis* are indistinguishable from one another in COI barcodes as a part of this species. In Central Asia, if our proxy types are correctly identified then the taxa *borochorensis* and *turkestanicus* Krüger (a junior homonym of *turkestanicus* Skorikov, 1910) are also indistinguishable from one another in COI barcodes as a part of this species. In Northern and Eastern Asia, the taxa *burjaeticus* and *albocinctus*, described previously as two separate species, are supported as parts of this species. In the Kuril Islands, the taxon *florilegus* has often been regarded as a separate species (Tkalcu, 1962; Sakagami & Ishikawa, 1969; Ito & Sakagami, 1980; Bertsch, 2009, but see Bertsch *et al.*, 2010a), whereas here it is interpreted as a semi-melanic part of this species. In North America, the taxon *moderatus* has usually been regarded as a separate species (Franklin, 1913; Scholl *et al.*, 1990; Bertsch, 2009, but see Milliron, 1971), whereas here (like Bertsch, 2010) it is interpreted as part of this species.

Cryptic species

Our study shows that in addition to the European cryptic species that are widespread beyond Europe, there are other putative species of the subgenus *Bombus s. str.* from outside Europe that are also cryptic by morphology, although reliably diagnosable from COI barcodes. These taxa have been regarded previously as parts of other species, because no unambiguous diagnostic morphological characters are known. Significantly, none of the putative species recognised here is a previously unnamed taxon (see Appendix). Even those taxa recognised for the first time as separate putative species from the COI-barcode data (*B. lantschouensis*, *B. minshanensis*), do have some morphological diagnosis, even if only in supposed differences in the colour patterns of the pile (Vogt, 1908; Bischoff, 1936). The challenge now is in

finding which among the described subtle morphological differences for different taxa are truly diagnostic of species supported from other evidence. Thus we see the next step lying in integrated taxonomy, using all available evidence from other DNA, pheromones (De Meulemeester *et al.*, 2011), morphology (Rasmont, 1984; Rasmont *et al.*, 1986) and perhaps other characters yet to be explored.

Some of the species of the subgenus *Bombus s. str.* worldwide that form cryptic groups with similar colour patterns of the pile are: (1) species with females with two yellow stripes and a white tail, including parts of the species: *B. cryptarum*, *B. jacobsoni*, *B. longipennis*, *B. lucorum*, *B. magnus*, *B. sporadicus* and *B. terrestris*; (2) species with females with an extra yellow band on the scutellum and often on metasomal tergum 1, including parts of the species: *B. cryptarum*, *B. lantschouensis*, *B. lucorum* and *B. sporadicus*; (3) species with females with similar but extensively white or grey colour patterns, including parts of the species: *B. lantschouensis*, *B. minshanensis* and *B. patagiatus*; and (4) species with females with two yellow bands and an orange-brown tail, including parts of the species: *B. hypocrita*, *B. patagiatus*, *B. terrestris* and (infrequently) *B. ignitus*. It remains to be seen whether there is evidence of regional convergence in pile colour patterns among these taxa (*cf.* Williams, 2007), or whether the similarities are purely the result of descent without modification.

Conclusions

We are far from clear and agreed decisions regarding the species of the subgenus *Bombus s. str.* COI barcodes, which have been extracted relatively easily from recent museum specimens, have enabled us for the first time to survey a priori problematic cryptic species in a unified analysis of many samples from throughout their distributions worldwide. By adopting a collaborative approach, we were able to sample all of these bumblebee species on a small budget, although this hides the far greater cost of the original field work to acquire specimens and the cost of maintaining the museum collections. It depends also on goodwill in the original countries, to permit the collecting and sequencing of specimens. We conclude primarily that results using GMYC analysis of COI barcodes provide one transparent and accountable interpretation of these cryptic species. Evidence from other genes is needed to test this. The voucher specimens of the modified putative species also need to be reviewed for morphological diagnostic characters, to try to establish keys that can be applied to the bulk of older museum specimens and even perhaps in field studies. Above all, we hope that a clarification of the taxonomy of the cryptic species will make it easier to develop commercial pollination from the most appropriate indigenous species, so that the introduction of invasive non-native species (or subspecies, Ings *et al.*, 2005) and their pathogens can be avoided. Clarification of taxonomy is also an essential pre-

requisite for investigating conservation needs and hence for implementing appropriate conservation actions.

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Appendix: Nomenclatural standards

Paul H. Williams

This appendix seeks to link selected names in the subgenus *Bombus s. str.* (= *Terrestribombus* Vegt) to our COI-barcode sequences. This is necessary because sequences are rarely available from the original type specimens.

Four hundred and eight published names for bees of the subgenus *Bombus s. str.* have been found for this review. Of these, 151 are available names in the species group (the remaining 257 names are unavailable names: mostly infrasubspecific names, together with incorrect subsequent spellings). The 52 names listed here are considered to include the oldest available names for the most divergent taxa and consequently include all of the valid species names.

The names are listed alphabetically. Each name is followed in parentheses by the name of the species to which it belongs according to the interpretation in this paper. On the next line is shown the original citation in its original form, with interpolations in brackets for the full name of the genus, subgenus or species, as implied by the context of the name in the original paper. Issues affecting the availability and application of the names are outlined, followed by explicit arguments for links between the original type specimens and the proxy voucher sequences used in this paper. Abbreviations for depositories of specimens are expanded in Table 1.

Informal proxies for the types are chosen (in order of preference) for: (1) matching morphology and colour pattern of the pile; (2) proximity of the sample site to the type locality; (3) full length COI-barcode sequences; (4) females and especially queens in good condition. These proxies are intended only for the purposes of this project, to facilitate the present link between true primary type specimens and COI barcodes for the sake of accountability. The proxies are not intended to have any formal nomenclatural status, or any longer-term persistent significance.

affinis (*affinis*)

B.[ombus] *affinis* Cresson, 1863: 103. [sic]

Type locality from original publication: ‘Canada’ and ‘New York’ (USA).

Type material: Lectotype worker from Canada (not a queen; ANSP) by designation of Cresson (1916: 110). Not seen, but taxon concept not in doubt.

Barcode proxy type: B#198 from a worker, from Pinery Provincial Park, Ontario, Canada (collected by A. Taylor, in 2009). This matches the usual concept of the taxon in colour pattern and morphology. Voucher specimen in the LP collection.

***africanus* (*terrestris*)**

Bombus [(*Terrestribombus*)] *terrestris*, Rasse *africanus* Vogt in Krüger, 1956: 91.

Availability: Krüger's (1951: 142) statements show that he intended 'geographische Rassen' to be equivalent to subspecies in rank (ICZN, 1999: Art. 45.6.4).

Type locality from original publication: 'Teberda', 'Tiemcen', 'Maison carré und Chemin des Crêtes' (Algeria).

Type material: syntype queens, workers, males (ZMA). Syntype worker (ZMA, 'Alger Chemin / des crêtes') examined. Taxon concept not in doubt.

Barcode proxy type: B#307 from a worker, from Bouira, Algeria (collected by L. Bendifallah, in 2005). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

***alaiensis* (?*lucorum*)**

Bombus [(*Terrestribombus*)] *lucorum* [var.] *alaiensis* Reinig, 1930: 107 no. 1.

Availability: Reinig (1930) used the term 'ssp.' as well as 'var.' in his publication, so 'var.' is deemed to be of infrasubspecific rank and the name is unavailable (ICZN, 1999: Art. 45.6.4). However, Krüger (1951: 195) subsequently used the name '*alaiensis* Reinig' at the subspecific rank of 'Rasse' (Krüger, 1951: 142), so that it is deemed to be available from the date of Reinig's original publication (ICZN, 1999: Art. 45.6.4.1).

Type locality from original publication: 'Gultscha (Alai)' (Gulcha, Kyrgyzstan), 'Kisil-beles (Alai)' (Kyrgyzstan), 'Taldik (Alai)' (Kyrgyzstan), 'Jaman-tal (Pamire)' (Tajikistan).

Type material: syntype queens and workers (?MNHU), syntype queens (ZMA). One queen (ZMA) agrees with the original description and carries the labels: (1) printed 'Alai, VI.28 / leg. Reinig'; (2) handwritten 'Taldik / 26.VI.3200 m'; (3) white printed 'Collectie / C. et O. Vogt / Acq. 1960'; (4) red printed 'LECTOTYPE [female] / *Bombus lucorum* / *alaiensis* / Reinig, 1930 / det. PH Williams 2011'. This queen, which lacks the right mid leg, is designated here as **LECTOTYPE** in order to reduce uncertainty in the identity and application of the name. Examined.

Barcode proxy type: B#40 from a worker, from close to Bishkek, Kyrgyzstan (collected by O. Tadauchi, in 2003). Because according to our results at least two morphologically closely similar species of *Bombus s. str.* occur in the broad region of the type locality (*B. cryptarum*, *B. lucorum*), associating sequenced specimens of either of these species with an unsequenced type specimen (*B. lucorum alaiensis*) has to be subjective and provisional. Available morphological diagnoses (Rasmont, 1984) are

difficult and are unproven outside Europe, but appear consistent with this interpretation. The lectotype shares with *B. lucorum* (cf. *B. cryptarum*): (1) clypeus with punctures in the medial ventral area less dense, smaller and shallower; (2) metasomal tergum 2 with punctures in the medial posterior area less dense with weaker intervening surface sculpturing. Specimen in the project voucher collection.

***albocinctus* (*cryptarum*)**

BOMBUS ALBOCINCTUS Smith, 1854: 397 no. 41.

Type locality from original publication: 'Kamtschatka' (Kamchatka, Russia).

Type material: holotype queen (BMNH) by monotypy (unnecessary lectotype designation by Tkalcu, 1974c: 32). Examined.

Barcode proxy type: B#482 from a queen, from Vestnik bay, Kamchatka, Russia (collected by A. Lelej and S. Storozhenko, in 1999). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

***armeniensis* (*cryptarum*)**

Bombus cryptarum [subsp.] *armeniensis* Rasmont, 1984: 138.

Type locality from original publication: 'Anatolie, Kars, Yalnizçam geçidi près de Ardahan' (Turkey).

Type material: holotype queen (ZSM) by original designation. Not seen, but taxon concept not in doubt.

Barcode proxy type: B#426 from a worker, from Sivas, Turkey (collected in 2010). This matches the usual concept of the taxon in colour pattern and morphology. Specimen in the project voucher collection.

***audax* (*terrestris*)**

Apis Audax Harris, 1776: 130 (not 137).

Type locality from original publication: England. It is uncertain where Harris collected, but it is presumed to be near where he lived, 'some distance from London' (Salmon, 2000).

Type material: original illustration (BMNH). Examined.

Barcode proxy type: B#556 from a queen, from Bromley, close to London, UK (collected by P. Williams, in 2011). This matches the usual concept of the taxon in colour pattern and morphology. Specimen in the project voucher collection.

***beickianus* (*lantschouensis*)**

[*Bombus* (*Bombus*)] *lucorum* [subsp.] *beickianus* Bischoff, 1936: 2.

Type locality from original publication: 'Siningebirge' (mountains near Xining, Qinghai, China), 'Tschili' (?Xili[gou], Qinghai, China).

Type material: lectotype queen (MNHU) by individual designation from the syntype series as 'holotype' (ICZN, 1999: Article 74.5) by Tkalcu (1967: 54), carrying the labels: (1) yellow printed 'Sining-Gebiet / Heitsuitse / Beick 1930'; (2) handwritten '*lucorum* / *beicki* n. subsp. C / [female] / det. Bischoff'; (3) red printed 'Typus'; (4) white printed 'Zool. Mus. / Berlin'; (5) red printed 'LECTOTYPE [female] / *Bombus lucorum* /

beickianus / Bischoff, 1936 / det. B Tkalcu 1967'; (6) white printed '[female] *Bombus* / (*Bombus*) / *lantschouensis* / det. PH Williams 2011'. This queen is complete. Examined.

Barcode proxy type: B#402 from a worker, from Xining, Qinghai, China (collected by P. Williams, in 2010). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

***borochorensis* (?cryptarum)**

Bombus [(*Terrestribombus*)] *magnus*, Rasse *borochorensis* Krüger, 1954: 273.

Availability: Krüger's (1951: 142) statements show that he intended 'geographische Rassen' to be equivalent to subspecies in rank (ICZN, 1999: Art. 45.6.4).

Type locality from original publication: 'Schlucht Burchan im Boro-Choro Gebirge' (Burchan gorge, interpreted as one of the northern valleys of the Burkhan river, in the western Borohoro mountains, Almaty, Kazakhstan).

Type material: syntype queens, workers, males (ZMA). Syntype queen (ZMA, 'Boro-Chorogeb / Schlucht Bur- / chan', labelled as lectotype by P. Rasmont, unpublished) examined.

Barcode proxy type: B#44 from a queen, from Sayram lake, Xinjiang, China (collected by A. Dawut, in 2002). Because according to our results at least two morphologically closely similar species of *Bombus* s. str. occur in the broad region of the type locality (*B. cryptarum*, *B. lucorum*), associating sequenced specimens of either of these species with an unsequenced type specimen (*B. magnus borochorensis*) has to be subjective and provisional. Available morphological diagnoses (Rasmont, 1984) are difficult and are unproven outside Europe, but appear consistent with this interpretation. The prospective lectotype shares with *B. cryptarum* (cf. *B. lucorum*): (1) clypeus with punctures in the medial ventral area denser, larger and deeper; (2) metasomal tergum 2 with punctures in the medial posterior area denser with stronger intervening surface sculpturing. Specimen in the project voucher collection.

***brevipilosus* (patagiatus)**

[*Bombus* (*Bombus*)] *patagiatus* [subsp.] *brevipilosus* Bischoff, 1936: 4.

Type locality from original publication: 'Hanka-See' (Khanka Lake, Primorsky, Russia), 'Korea'.

Type material: syntypes (?MNHU). Not seen, but taxon concept not in doubt.

Barcode proxy type: B#380, from a male, from Xinkai, near Khanka Lake, in Heilongjiang, China (collected in 2008). This matches the usual concept of the taxon in colour pattern and morphology. Specimen in the project voucher collection.

***burjaeticus* (?cryptarum)**

Bombus [(*Terrestribombus*)] *burjaeticus* Krüger, 1954: 277 no. 3.

Type locality from original publication: 'Kul'skiy, Respublika Buryatiya, Russia).

Type material: syntype queens, workers, males (ZMA). Syntype queen (ZMA, 'Kulskoje', labelled as lectotype by P. Rasmont, unpublished) examined.

Barcode proxy type: B#267 from a queen, from by the Tuul River, Ulaanbaatar, Mongolia (collected by J. Halada, in 2003), the closest available sample. Because according to our results at least two morphologically closely similar species of *Bombus* s. str. occur in the broad region of the type locality (*B. cryptarum*, *B. lucorum*), associating sequenced specimens of either of these species with an unsequenced type specimen (*B. burjaeticus*) has to be subjective and provisional. Available morphological diagnoses (Rasmont, 1984) are difficult and are unproven outside Europe, but appear consistent with this interpretation. The prospective lectotype shares with *B. cryptarum* (cf. *B. lucorum*): (1) clypeus with punctures in the medial ventral area denser, larger and deeper; (2) metasomal tergum 2 with punctures in the medial posterior area denser with stronger intervening surface sculpturing. In addition, the prospective lectotype has the pile with: (1) an extension of the yellow on the side of the thorax (episternum, shared by both *B. lucorum* and *B. cryptarum* in this part of Asia, consequently uninformative); and (2) often (but not always) a greenish tint of the yellow, although the precise hue of the yellow can be unreliable as a taxonomic character in this group of bees (Bertsch *et al.*, 2004). Specimen in the project voucher collection.

***canariensis* (terrestris)**

BOMBUS TERRESTRIS L., var. *canariensis* Pérez, 1895: 191 no. 2.

Type locality from original publication: 'Canaria et Ténérife' (Gran Canaria and Tenerife, Spain).

Type material: lectotype queen (MNHN) by designation of Erlandsson (1979: 189). Not seen, but taxon concept not in doubt.

Barcode proxy type: B#434 from a queen, from Gran Canaria (collected by P. Sima, in 2008). This matches the usual concept of the taxon in colour pattern and morphology. Specimen in the project voucher collection.

***cryptarum* (cryptarum)**

[*APIS*] *cryptarum* Fabricius, 1775: 379 no. 6.

Type locality from original publication: 'Habitat Hafniae' (Copenhagen, Denmark, according to Løken, 1973: 40).

Type material: stated originally to be in 'Mohr.'. Lectotype queen (KU) by designation of Løken (1966:199). Not seen, but taxon concept not in doubt.

Barcode proxy type: B#169, from a queen, from southern Denmark (collected by H. Schmidt, in 2001). This matches the usual concept of the taxon in colour pattern and morphology. Specimen in the project voucher collection.

***czerskianus* (sporadicus)**

[*Bombus*] *Tb.*[(*Terrestribombus*)] *terrestris czerskianus* Vogt, 1911: 56.

Type locality from original publication: 'Chankasee. Mongolei.' (Khanka Lake, Primorsky, Russia; and Mongolia).

Type material: syntype workers (ZMA). Syntype worker (ZMA, 'Fluss / Tjutiché', labelled as lectotype by T. De Meulemeester and P. Rasmont, unpublished) examined.

Barcode proxy type: B#355 from a worker, from Primorsky, Russia (collected by M. Quest, in 2001). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

***dalmatinus* (terrestris)**

[*Bombus* (*Leucobombus*) *terrestris* var.] *Dalmatinus* Dalla Torre, 1882: 26.

Type locality from original publication: 'Mittelmeerküsten . . . Fiume, Livorno, Sizilien, Spalato und Ragusa, Athen, Amasia und Elisabethpol' (Mediterranean coast).

Type material: syntype series (location unknown). Not seen, but taxon concept not in doubt.

Barcode proxy type: B#428 from a queen, from close to Siena, Italy (collected by P. Williams, in 1995). This matches the usual concept of the taxon in colour pattern and morphology. Specimen in the project voucher collection.

***flavoscutellaris* (magnus)**

Bombus terrestris L. var. nov. *flavoscutellaris* Trautmann & Trautmann, 1915: 96.

Type locality from original publication: 'in der Umgebung von Eberswalde' (in the environs of Eberswalde, Brandenburg, Germany).

Type material: syntype queens (?MNHU and ZMA according to Krüger, 1954: 269). Not seen, but taxon concept not in doubt.

Barcode proxy type: B#183 from a queen, from eastern Denmark (collected by H. Schmidt, in 2001). This matches the usual concept of this taxon in colour pattern and morphology. Specimen in the project voucher collection.

***florilegus* (cryptarum)**

Bombus ([*Bombus*] s. str.) *florilegus* Panfilov, 1956: 1334 no. 12.

Type locality from original publication: Islands of Simushir and Iturup (Kuril archipelago, Russia).

Type material: syntype workers and males (?ZMMU). Not seen, but taxon concept not in doubt.

Barcode proxy type: B#215 from an individual of unknown sex or caste. A COI-barcode sequence available (GenBank AF279487) from one of the type localities, Etorofu Island (Iturup), is used here. Specimen location unknown.

***franklini* (franklini)**

Bremus (*Bombus*) *franklini* Frison, 1921: 147.

Type locality from original publication: 'Nogales, Arizona' but actually Oregon (USA) according to Thorp (1970).

Type material: holotype queen (INHS) by original designation. Examined.

Barcode proxy type: B#196 from a male, from Mount Ashland, Oregon, USA (collected by R. Thorp, in 2003). This is the only COI sequence (GenBank AY694097) currently available for this species, which may now be extinct (Buchmann *et al.*, 2008). Voucher specimen in the NMS.

***ganjsuensis* (patagiatus)**

B.[ombus] ikonnikovi [var.] *ganjsuensis* Skorikov, 1913: 172.

Availability: Skorikov (1913) does not make the status of this taxon explicit as 'var.' or 'ab.', as he does elsewhere in this paper (with the exception of one other case). But because he describes this taxon as differing in what he considered significant characters from *B. ikonnikovi* s. str., this is interpreted as implying the status of variety, and is deemed to be of subspecific rank. The name is therefore available (ICZN, 1999: Art. 45.6.4).

Type locality from original publication: 'Südöstlicher Gan-su' (Gansu, China).

Type material: Holotype queen (ZISP) by monotypy. Examined.

Barcode proxy type: B#383 from a queen, from Gansu, China (collected in 2007). This matches the usual concept of the taxon in colour pattern and morphology. Specimen in the project voucher collection.

***gilgitensis* (tunicatus)**

Bombus gilgitensis Cockerell, 1905: 223.

Type locality from original publication: 'Gilgit, Kashmir' (India).

Type material: holotype queen (BMNH) by monotypy. Examined.

Barcode proxy type: B#186 from a queen, from Kargil, Kashmir, India (collected by R. Raina, in 2007). This queen matches the type specimen in colour pattern and morphology. Voucher specimen in the R. Raina personal collection, Srinagar, Kashmir, India.

***hypocrita* (hypocrita)**

B.[ombus] IGNITUS Sm., var. *HYPOCRITA* Pérez, 1905: 30.

Availability: Pérez (1905) uses only the term 'var.' in his publication without any reference to a higher rank of subspecies, so 'var.' is deemed to be of subspecific rank and the name available (ICZN, 1999: Art. 45.6.4).

Type locality from original publication: 'Japon' (in the title 'le Japon central', ?Honshu, Japan) and 'Transbaïkalie' (Russia).

Type material: three syntype workers from Japan and one syntype worker from Transbaikal (MNHN, examined). Tkalcu (1962: 89) plotted a map of the records, including Pérez's record for the Transbaikal region (also listed among the material he examined on his p. 92). Sakagami & Ishikawa (1969: 185) considered that Pérez's material is likely to be a mixed syntype series, considered alternatives, and preferred the interpretation that *hypocrita* is based on material from Honshu (their p. 170). This interpretation was later agreed by Tkalcu (in Sakagami & Ishikawa, 1972: 609), who was then uncertain whether the material described by Pérez as coming from Transbaikal did originate from there (it may have been mislabelled). Our COI results have so far shown no evidence that the Japanese species

occurs in Transbaikal (our nearest samples are for Korea and Primorsky), although all four syntypes appear to belong to this species. One worker (MNHN) agrees with the original description and carries the labels: (1) printed 'Muséum Paris / EY6003'; (2) printed 'Japan'; (3) printed 'MUSEUM PARIS / COLL. J. PÉREZ 1915'; (4) red printed 'LECTOTYPE worker / *Bombus ignitus* / *hypocrita* / Pérez, 1905 / det. PH Williams 2011'. This worker, which is relatively complete with only a small hole on the left side of the thorax, is designated here as **LECTOTYPE** in order to reduce uncertainty in the identity and application of the name. Examined.

Barcode proxy type: B#550 from a queen, from central Honshu, Japan (collected by S. Martin, in 1989). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

ignitus (*ignitus*)

Bombus ignitus Smith, 1873: 206 no. 3.

Type locality from original publication: 'Hakodadi' (Japan).

Type material: holotype queen (BMNH) by monotypy. Examined.

Barcode proxy type: B#207 from a worker, from Kyushu, Japan (collected by K. Takamizawa, in 2009). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

iranicus (*cryptarum*)

Bombus [(*Terrestribombus*)] *magnus*, Rasse *iranicus* Krüger, 1954: 273.

Availability: Krüger's (1951: 142) statements show that he intended 'geographische Rassen' to be equivalent to subspecies in rank (ICZN, 1999: Art. 45.6.4).

Type locality from original publication: 'Elburs, aus 2000 m Höhe' (Elburz, Iran).

Type material: syntype queens (?MNHU, referred to by Krüger, 1954). Not seen, but taxon concept not in doubt.

Barcode proxy type: B#31 from a queen, from Walazir, Ardabil, Iran (collected by A. Monfared, in 2006). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

jacobsoni (*jacobsoni*)

B.[ombus] lucorum (L.) subsp. *jacobsoni* Skorikov, 1912: 610.

Type locality from original publication: 'Passhöhe Zodzhi-La' (Zoji La, upper Sind valley, Kashmir, India) and 'Kashmir, Maru, Farga-bad' (Wardwan valley, Kashmir, India).

Type material: syntype queen (ZISP) examined, syntype queen (BMNH accessioned in 1934, according to the NHM accession register entry 560, as part of a collection of 90 Hymenoptera specimens from Russia and Siberia sent as an exchange of material) examined. Podbolotskaya's manuscript lectotype designation (Williams, 1991: 82) has never been published. This queen from Fargabad (ZISP) agrees with the original description and carries the labels: (1) handwritten in Russian '[lo-

cality: Fargabad, river Dar -/davan. Maru, Kashmir/P. Trubetskoi, 19.V.1910]'; (2) handwritten in pencil '*jacobsoni*'; (3) red printed '*Lectotypus Bombus/lucorum* subsp./*jacobsoni* Skor./design. Podbolotsk.' (unpublished); (4) red printed 'LECTOTYPE [female]/*Bombus lucorum* ssp./*jacobsoni*/Skorikov, 1912/det. PH Williams 2011'. This queen, which is complete, is designated here as **LECTOTYPE** in order to reduce uncertainty in the identity and application of the name. Examined.

Barcode proxy type: B#532 from a queen, from Dawar, Kashmir, India (collected by R. Raina, in 2007). This matches the type specimen in colour pattern and morphology. Two sequences were obtained from this specimen, both just 425 nucleotides long, but identical.

lantschouensis (*lantschouensis*)

B.[ombus] lucorum L. var. *lan-tschoúensis* Vogt, 1908: 101 no. 3.

Availability: Vogt (1908) uses only the term 'var.' in his publication without any reference to a higher rank of subspecies, so in a publication of this date 'var.' is deemed to be of subspecific rank and the name available (ICZN, 1999: Art. 45.6.4). The use of diacritic marks and hyphens is an incorrect original spelling that has to be corrected (ICZN, 1999: Art. 32.5.2).

Type locality from original publication: 'Lan-tschoú' (Lanzhou, Gansu, China).

Type material: syntype queens, workers, males (ZMA). Syntype queen (ZMA, 'Lantschou', labelled as lectotype [of 'LAND-SCHOUENSIS'] by P. Rasmont, unpublished) examined.

Barcode proxy type: B#422 from a worker, from close to Lanzhou, Gansu, China (collected by P. Williams, in 2010). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

longipennis (*longipennis*)

Bombus pratorum var. *longipennis* Friese, 1918: 83 no. 13.

Availability: Friese (1918) uses only the term 'var.' in his publication without any reference to a higher rank of subspecies, so 'var.' is deemed to be of subspecific rank and the name available (ICZN, 1999: Art. 45.6.4). Furthermore, Tkalcu (1974a: 324) subsequently used this name as the valid name for a species, *Bombus longipennis* Friese, so that it would also be seen to be available from the date of Friese's original publication (ICZN, 1999: Art. 45.6.4.1).

Type locality from original publication: 'Sikkim' (India, but possibly actually the neighbouring part of Xizang, China).

Type material: lectotype worker (MNHU) by designation of Williams (1991: 82). Examined.

Barcode proxy type: B#490 from a worker, from southern Xizang, China (collected by Z. Miao, in 2008), close to the Sikkim border. No recent bumblebee material is accessible from Sikkim for sequencing. The recent worker specimen available from just across the border in Xizang comes closest to this taxon among the material available in that it has the yellow band on T2 narrowly interrupted by black pile medially. Two characters do not agree with the original description (the yellow anterior thoracic dorsal band is only notched and not interrupted medially; and

white pile is present posteriorly narrowly on T4 as well as on T5) but they do agree with the lectotype worker. Specimen in the project voucher collection.

lucorum (*lucorum*)

APIS *lucorum* Linnaeus, 1761: 425 no. 1716. [sic]

Type locality from original publication: ‘Habitat in Lucis’ (Uppland, Sweden, according to Løken, 1973: 40).

Type material: lectotype male (LSL) by designation of Day (1979: 66, but referred to erroneously in ICZN Opinion 1828 [1996: 64] as a neotype). According to Day (1979), this specimen was examined and the current taxonomic interpretation agreed by Løken (1973: 40) and subsequently by Løken *et al.* (1994: 233 [ICZN Case 2638]). Examined.

Barcode proxy type: B#367 from a male, from Linnaeus’s Hammarby, Sweden (collected by B. Cederberg, in 2010), the presumed type locality. This male is of the ‘blonde’ colour form conventionally associated with this species in Europe (Bertsch, 2009). Specimen in the project voucher collection.

lusitanicus (*terrestris*)

Bombus [(*Terrestribombus*)] *terrestris*, Rasse *lusitanicus* Krüger, 1956: 78.

Availability: Krüger’s (1951: 142) statements show that he intended ‘geographische Rassen’ to be equivalent to subspecies in rank (ICZN, 1999: Art. 45.6.4).

Type locality from original publication: ‘Castello da Vide’, ‘St. Thirso da Famalicæ’, ‘Sierra de Estrela’, ‘St. Martinho’ (Portugal).

Type material: syntype queens, workers, males (ZMA). Syntype queen (ZMA, ‘Castello da / Vide’) examined. Taxon concept not in doubt.

Barcode proxy type: B#437 from a queen, reared from another collected in Perpignan, France (by P. Sima, in 2008). This matches the type specimens in colour pattern and morphology. Specimen in the project voucher collection.

luteostriatus (*magnus*)

Bombus [(*Terrestribombus*)] *magnus*, Rasse *luteostriatus* Krüger, 1954: 272.

Availability: Krüger’s (1951: 142) statements show that he intended ‘geographische Rassen’ to be equivalent to subspecies in rank (ICZN, 1999: Art. 45.6.4).

Type locality from original publication: ‘Gèdres’ (France).

Type material: syntype queens, male (ZMA). Syntype queen (ZMA, ‘Gèdres’) examined. Taxon concept not in doubt.

Barcode proxy type: B#320 from a queen, from Soria, Spain (collected by I. Yarrow and E. Yarrow, in 1965). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

maderensis (*terrestris*)

Bombus maderensis Erlandsson, 1979: 191.

Type locality from original publication: ‘Madeira, Funchal’ (Portugal).

Type material: holotype queen (NHR) by original designation. Not seen, but taxon concept not in doubt.

Barcode proxy type: B#214 from an individual of unknown sex or caste. A COI sequence is available (GenBank AF18112) from Madeira. Specimen location unknown.

magnus (*magnus*)

[*Bombus* (*Terrestribombus*) *lucorum* Forma] *magnus* Vogt, 1911: 56.

Availability: Vogt’s (1911: 50 footnote) statements show that he intended ‘Varietas geographica’ to be equivalent to subspecies in rank and ‘Aberratio extrema’ to be of infrasubspecific rank. He uses ‘Forma’ for taxa that he could not assign to either rank with certainty and these are treated as being of subspecific rank (ICZN, 1999: Art. 45.6.4). Furthermore, Krüger (1951: 143, 1954: 264) subsequently used this name as the valid name for a species, *Bombus magnus* Vogt, so that it is now deemed to be of subspecific rank and available from the date of Vogt’s original publication (ICZN, 1999: Art. 45.6.4.1).

Type locality from original publication: ‘in Nordschottland und auf den Orkneyinseln’ (in northern Scotland and on the Orkney Isles, UK).

Type material: syntype queens, workers, males (ZMA). In Britain, using morphological characters, Alford (1975) mentions *B. magnus* in his text as occurring ‘in the north of Scotland’, although the included Bumblebee Distribution Maps Scheme map shows records including the mainland, Hebrides and Orkneys (included also in the map in Rasmont, 1984). Baker (1996) records it from Sutherland. In contrast, from COI results, while the presence of *B. magnus* on the mainland is supported (Murray *et al.*, 2008; Bertsch, 2009), there is no confirmation of its occurrence in the Orkneys, where *B. cryptarum* is now shown to occur (Murray *et al.*, 2008; Bertsch, 2009). Sample sizes are still small, but it is possible that *B. magnus* does not occur on the Orkneys now, and presumably not at the time when Vogt’s specimens were collected. This emphasises the importance of relating names to COI groups. If a lectotype were selected from among Vogt material from the Orkneys, then it is likely that *B. magnus* would be a junior synonym of *B. cryptarum*. But hopefully it should be possible to select a lectotype from among the Vogt material from the northern Scottish mainland on the basis of morphology that can be related with confidence to the COI taxon distinct from *B. cryptarum*, so that the current usage of *B. magnus* can be maintained. Syntype queen (ZMA, ‘Thurso’, labelled as lectotype by P. Rasmont, unpublished) examined.

Barcode proxy type: B#202 from a queen, from Torrisdale Bay, Sutherland, northern Scotland, UK (collected by R. Dawson, in 2010). This queen matches the Thurso syntype (prospective lectotype) in colour pattern and morphology. Specimen in the project voucher collection.

malaisei (*sporadicus*)

[*Bombus* (*Terrestribombus*)] *terrestris* L. [subsp.] *malaisei* Bischoff, 1930: 4.

Type locality from original publication: 'Klutschi' (Kamchatka, Russia).

Type material: holotype queen (NHR) by original designation. Not seen, but taxon concept not in doubt.

Barcode proxy type: B#492 from a queen, from Vestnik bay, Kamchatka, Russia (collected by A. Lelej and S. Storozhenko, in 1999). This matches the usual concept of the taxon in colour pattern and morphology. Specimen in the project voucher collection.

***mckayi* (*occidentalis*)**

BOMBUS MCKAYI Ashmead, 1902: 125. [sic]

Type locality from original publication: 'Nushagak River' (Alaska, USA).

Type material: holotype queen (USNM) by original designation ('Cat. No. 5719'). Examined.

Barcode proxy type: B#498 from a queen, from Palmer, Alaska, USA (collected in 2009). This matches the usual concept of the taxon in colour pattern and morphology. Voucher specimen in the LP collection.

***minshanensis* (*minshanensis*)**

[*Bombus* (*Bombus*)] *patagiatus* [subsp.] *minshanensis* Bischoff, 1936: 3.

Type locality from original publication: from dates given and comparison with Sjöstedt & Hummel (1932): 'Min-chow' (Minxian or Mizhou), 'Taggama' (unknown), 'Tang-chang' (Tanchang), 'Drakana' (?Zhagana; all sites in the Minshan range, Gansu, China).

Type material: syntype queens, male (MNHU). One queen (labelled 'juni', presumably from 27 or 30/06/[1930] according to the original description, and therefore presumably from 'Min-chow' according to Sjöstedt & Hummel, 1932) agrees with the original description and bears the labels: (1) white printed 'Kina/S. Kansu'; (2) white printed 'juni'; (3) white printed 'Sven Hedins/Exp. Ctr. Asien/Dr Hummel'; (4) handwritten '*patagiatus/minshanensis*/n. subsp. [female]/det. Bischoff'; (5) red printed 'Para-/Typus' (the 'Para' handwritten); (6) white printed 'Zool. Mus./Berlin'; (7) red printed 'LECTOTYPE [female]/*Bombus patagiatus/minshanensis*/Bischoff, 1936/det. PH Williams 2011'. This queen, which is complete, is designated here as **LECTOTYPE** in order to reduce uncertainty in the identity and application of the name. Examined.

Barcode proxy type: B#246 from a worker, from Hezuo, southern Gansu, China (collected by P. Williams, in 2009). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

***minshanicola* (*longipennis*)**

[*Bombus* (*Bombus*)] *terrestris* [subsp.] *minshanicola* Bischoff, 1936: 2.

Type locality from original publication: from dates given and comparison with Sjöstedt & Hummel (1932): 'Shi-men' (Shimen), 'Drakana' (?Zhagana), 'Wutsena' (?Waina), 'Liang-chia-pa'

(Liangshui), 'Tjeggala' (?Tielou, all sites in the Minshan range, Gansu, China).

Type material: part of the original syntype series is believed to have been returned to the NHR but cannot now be located (H. Vårdal in Bertsch, 2010). A syntype worker (MNHU) labelled 29/07/[1930] (which would correspond to 'Drakana' according to Sjöstedt & Hummel, 1932) agrees with the original description and is the only specimen seen that was collected at a date given for the original syntypes. This worker bears the labels: (1) handwritten '*terrestris/minshanicola*/n. subsp. [worker]/det. Bischoff'; (2) white printed 'Sven Hedins/Exp. Ctr. Asien/Dr Hummel'; (3) white printed 'Kina/S. Kansu'; (4) handwritten pencil '29/7'; (5) red printed 'Para-/Typus' (the 'Para' handwritten); (6) handwritten '2 Beine au/BERTSCH für/DNA-unters./17.08.2010'; (7) white printed 'Zool. Mus./Berlin'; (8) red printed 'LECTOTYPE [worker]/*Bombus terrestris/minshanicola*/Bischoff, 1936/det. PH Williams 2011'. This worker, which lacks the right mid and hind legs, is designated here as **LECTOTYPE** in order to reduce uncertainty in the identity and application of the name. Examined.

Barcode proxy type: B#54 from a queen, from Zhagana, Minshan, southern Gansu, China (collected by P. Williams, in 2009), one of the likely syntype localities. Specimen in the project voucher collection.

***moderatus* (*cryptarum*)**

[*Bombus*] *moderatus* Cresson, 1863: 109 no. 22.

Nomenclature: replacement name for *B. modestus* Cresson (1863: 99), a junior primary homonym in *Bombus* of *Bombus modestus* Eversmann (1852: 134) (recognized by Cresson, 1863: 109, but as a junior primary homonym in *Bombus* of *Bombus modestus* Smith, 1861: 153).

Type material: see notes on *B. modestus*.

Barcode proxy type: B#222 from a queen, from Rosie Creek, Alaska (collected in 2009), close to the Yukon. This matches the usual concept of the taxon in colour pattern and morphology. Voucher specimen in the LP collection.

***modestus* (*cryptarum*)**

B.[*ombus*] *modestus* Cresson, 1863: 99 no. 22. [sic]

Nomenclature: junior primary homonym in *Bombus* of *Bombus modestus* Eversmann (1852: 134). See notes on *B. moderatus* as a replacement name.

Type locality from original publication: 'Youcon River, Arctic America' (?Alaska, USA).

Type material: holotype queen (ANSP) by monotypy. Not seen, but taxon concept not in doubt.

Barcode proxy type: see notes on *B. moderatus*.

***mongolicus* (?*lucorum*)**

Bombus [(*Terrestribombus*)] *magnus*, Rasse *mongolicus* Krüger, 1954: 276.

Availability: Vogt's (1909: 42) *B. lucorum* ab. *mongolicus* was published at infrasubspecific rank and is therefore unavailable (ICZN, 1999: Art. 45.6.2). Vogt's (1911: 56) *B. lucorum* var. *mongolicus* was published without description or indication and is therefore unavailable (ICZN, 1999: Art. 12.2). Skorikov's (1933: 57) *B. lucorum mongolicus* was published without description or indication (with only a reference to Vogt, 1911, which contains no description) and is therefore unavailable (ICZN, 1999: Art. 13.1.2). Krüger's (1954: 276) *B. magnus* Rasse *mongolicus* was published referring to Vogt, but without giving a specific bibliographic reference or date. However, Krüger (1954: 276) did give a description that differentiates the taxon. Krüger's (1951: 142) statements show that he intended 'geographische Rassen' to be equivalent to subspecies in rank (ICZN, 1999: Art. 45.6.4). Therefore the name *mongolicus* is considered available from Krüger's (1954) publication (ICZN, 1999: Art. 13.1.1).

Nomenclature: junior primary homonym in *Bombus* of *Bombus hortorum* var. *mongolicus* Friese (1916: 110) and a junior secondary homonym in *Bombus* of *Lapidariobombus alagesianus* ssp. *mongolicus* Skorikov (1931: 226).

Type locality from original publication: 'nördlichen [sic] Mongolei' (northern Mongolia). This taxon was first collected as a series of syntype queens, workers and a male, in 1892 by H. Leder in northern Mongolia (lectotype label: 'N. Mongolei/Leder 92'). Leder's route is described by Kerzhner (1972). Although there is no direct information about where along this route these specimens were taken, from the locality given it is likely to have been from the section of the route between Kyakhta and Ulaanbaatar.

Type material: lectotype queen (ZMA) by designation of Williams (1991: 82; also labelled as lectotype by B. Tkalcu, unpublished). Examined.

Barcode proxy type: B#270 from a worker, from close to Ulaanbaatar, Mongolia (collected by J. Halada, in 2003). Because according to our results at least two morphologically closely similar species of *Bombus* s. str. occur in the broad region of the type locality (*B. cryptarum*, *B. lucorum*), associating sequenced specimens of either of these species with an unsequenced type specimen (*B. magnus mongolicus*) has to be subjective and provisional. Available morphological diagnoses (Rasmont, 1984) are difficult and are unproven outside Europe, but appear consistent with this interpretation. The prospective lectotype shares with *B. lucorum* (cf. *B. cryptarum*): (1) clypeus with punctures in the medial ventral area less dense, smaller and shallower; (2) metasomal tergum 2 with punctures in the medial posterior area less dense (but denser and larger than for European *B. lucorum*) with weaker intervening surface sculpturing. In addition, the prospective lectotype has the pile with: (1) an extension of the yellow on the side of the thorax (episternum, shared by both *B. lucorum* and *B. cryptarum* in this part of Asia, consequently uninformative); and (2) a bright pale yellow with a whitish sheen giving an ochre impression (i.e. not greenish tint), although the precise hue of the yellow can be unreliable as a taxonomic character in this group of bees (Bertsch *et al.*, 2004). Specimen in the project voucher collection.

occidentalis (*occidentalis*)

Bombus occidentalis Greene, 1858: 12 no. 2.

Type locality from original publication: 'Fort Vancouver' (British Columbia, Canada) and 'Puget's Sound' (Washington, USA).

Type material: syntype females and male, lost according to Milliron (1971). However, the taxon concept is not in doubt, so the designation of a neotype would be unjustified (ICZN, 1999: Art. 75.1).

Barcode proxy type: B#329 from a worker, from Washington, USA (close to Vancouver, collected by R. Jacobson, in 1987). This matches the usual concept of this taxon in colour pattern and morphology. Specimen in the project voucher collection.

patagiatus (*patagiatus*)

B.[ombus] patagiatus Nylander, 1848: 234 no. 16.

Type locality from original publication: 'E Sibiria' (eastern Siberia, Russia), precise locality unknown (Tkalcu, 1967: 50).

Type material: presumed lost (Tkalcu, 1967: 50). However, the taxon concept is not in doubt, so the designation of a neotype would be unjustified (ICZN, 1999: Art. 75.1).

Barcode proxy type: B#342 from a worker, from Novosibirsk, Russia (collected by A. Byvaltsev, in 2006). This matches the usual concept of this taxon in colour pattern and morphology. Specimen in the project voucher collection.

proximus (*occidentalis*)

B.[ombus] proximus Cresson, 1863: 98 no. 21. [sic]

Type locality from original publication: 'Utah' (USA).

Type material: holotype worker (USNM, no image available) by monotypy. Not seen, but taxon concept not in doubt.

Barcode proxy type: B#90 from a queen, from Colorado, USA (collected by J. Gibbs, in 2007). This matches the usual concept of the taxon in colour pattern and morphology. Voucher specimen in the LP collection.

pseudosporadicus (*lantschouensis*)

[*Bombus* (*Bombus*) *lucorum* subsp.] *pseudosporadicus* Bischoff, 1936: 2.

Type locality from original publication: 'Tsingshuiho, Sui Yuan' (Qingshuihe, Huhehaote, Neimenggu, China).

Type material: lectotype queen (MNHU) by individual designation from the syntype series as 'holotype' (ICZN, 1999: Article 74.5) by Tkalcu (1967: 54), carrying the labels: (1) white printed 'Tsingshuiho / Sui Yuan / China'; (2) handwritten '*lucorum* / *pseudospora* - / *dicus* n. subsp. / det. Bischoff [female]'; (3) red printed 'Typus'; (4) handwritten 'LECTOTYPE / *Bombus lucorum* / *pseudospora* - / *dicus* Tkalcu det. / Bischoff [female]' (unpublished, except as above); (5) white printed 'Zool. Mus. / Berlin'; (6) red printed 'LECTOTYPE [female] / *Bombus lucorum* / *pseudosporadicus* / Bischoff, 1936 / det. B. Tkalcu 1967'; (7) white printed '[female] *Bombus* / (*Bombus*) / *lantschouensis* / det. PH Williams 2011'. This queen lacks part of the right antennal flagellum. Examined.

Barcode proxy type: B#6 from a worker, from Yingxian, Shanxi, China (collected by P. Williams, in 2007), the closest available sample. This matches the concept of this taxon in colour pattern and morphology. Because *pseudosporadicus* is closely similar to *lantschouensis* and not geographically discrete, only the suggested proxy is labelled *pseudosporadicus*. Specimen in the project voucher collection.

***reinigi* (*longipennis*)**

Bombus reinigi Tkalcu, 1974a: 322.

Type locality from original publication: 'W-Nepal, Ringmo/Phoksumdo-See . . . 3700 m' (Nepal).

Type material: holotype queen (NMS) by original designation. Examined.

Barcode proxy type: B#51 from a queen, from 'Churta' (Chauta), Nepal (collected by F. Creutzburg, in 2007). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

***reinigianus* (*cryptarum*)**

Bombus cryptarum [subsp.] *reinigianus* Rasmont, 1984: 137.

Type locality from original publication: 'Yougoslavie, Makedonija, Titov Vrh, Šar planina' (Macedonia).

Type material: holotype queen (ZSM) by original designation. Not seen, but taxon concept not in doubt.

Barcode proxy type: B#584 from a queen, from S-chanf, Switzerland (collected in 2011). This matches the usual concept of the taxon in colour pattern and morphology. Specimen in the project voucher collection.

***sapporoensis* (*hypocrita*)**

BOMBUS SAPPOROENSIS Cockerell, 1911: 641.

Type locality from original publication: 'Japan'.

Type material: holotype queen (USNM) by original designation ('Cat. No. 13425'). Examined (and images available online).

Barcode proxy type: B#204 from a queen, from Hokkaido, Japan (collected by S. Takahashi, in 2010). This matches the type specimen in colour pattern and morphology (although the type specimen has less extensive pale pile on the scutellum and on metasomal tergum 1 than the general interpretation placed on this taxon by Sakagami & Ishikawa, 1972). Specimen in the project voucher collection.

***sporadicus* (*sporadicus*)**

B.[ombus] sporadicus Nylander, 1848: 233 no. 15.

Type locality from original publication: 'Ostrobothnia' (Ostrobothnia, Finland) and 'Uleåborg' (Oulu, Finland).

Type material: lectotype worker (ZMH) by designation of Tkalcu (1967: 44). Not seen, but taxon concept not in doubt.

Barcode proxy type: B#429 from a worker, from Kopparberg, Sweden (collected by P. Williams, in 2006). This matches the usual concept of the taxon in colour pattern and morphology. Specimen in the project voucher collection.

***terrestricoloratus* (*cryptarum*)**

Bombus [(*Terrestribombus*)] *lucorum*, Rasse *terrestricoloratus* Krüger, 1951: 195.

Availability: Krüger's (1951: 142) statements show that he intended 'geographische Rassen' to be equivalent to subspecies in rank (ICZN, 1999: Art. 45.6.4).

Type localities from original publication: (1) 'Tunkun Sajan' (Sayan, Russia), (2) 'Nordtibat (5000 m)' (northern Tibet [?plateau]), and (3) 'Tibetanische Grenzberge' (mountains bordering Tibet [?plateau]).

Type material: this taxon was described from one syntype from Sayan and two from 'Tibet' (ZMA). The title above the description ('Das Ethnos des nördlichen Tibet') implies that Krüger intended the taxon to be based primarily on the Tibetan specimens rather than on the Sayan specimen. Before 1949, the term 'Tibet' was often used in a broader sense for much of what is now referred to as the Qinghai-Tibetan plateau, rather than for the current Chinese autonomous region of Xizang (Tibet) in the narrower sense. Renewed sampling efforts on the plateau in the last decade (especially from surveys by J. An, J. Huang and Y. Tang, see Williams *et al.*, 2010) have found bees of *Bombus s. str.* with two yellow stripes and a white tail only along the southern, eastern and north-eastern fringes of the plateau. The only areas where these bees have been found on the northern edge of the plateau so far are in the mountain ranges just north (Qilian Shan etc.) of Qinghai Hu (Lake Kuku Nor) in the north-east. All of those bees differ from the two Tibetan syntypes of *terrestricoloratus* Krüger in a range of characters (contrary to Tkalcu, 1974a: 324, see *minshanicola* Bischoff). It is therefore likely that the Tibetan specimens of *terrestricoloratus* Krüger originated from elsewhere. One possibility is in the high mountains further to the west, in the Altun Shan or Kunlun Shan ranges (Xinjiang). The labels on the specimens ('Nordtibat / Gebirge / 5000 m' and 'Tibet. / Grenzberge') are consistent with this, assuming that the information is correct. Despite the very few expeditions that are known to have collected bumblebees from these areas, so far it has not been possible to identify the origin of this material. Therefore no locality is shown on our map. A quick search of the IZB collection found no specimens that agree with the syntypes. The darker queen (possibly from the north of the Tibetan plateau) has: the pile of the head excluding the labrum and the mandibles entirely black (neither of the syntypes has obvious grey pile on the face); the yellow thoracic collar has black hair intermixed throughout and as a strong lateral black S anterior to the wing base, the yellow extending only just beyond the S; metasomal tergum 2 has the yellow band broad with only a very narrow posterior black band; and terga 4–5 are almost entirely white. It agrees with the original description and bears the labels: (1) handwritten pencil on squared paper 'Nordtibat / Gebirge / 5000 m'; (2) white printed 'Collectie / C. et O. Vogt / Acq. 1960'; (3) white printed 'Coll. Zoölogisch / Museum / Amsterdam'; (4) red printed 'Bombus / lucorum terrestricoloratus / Krüger, 1951 / ZMAN type HYME.0277.2'; (5) printed 'Terrestribombus / cryptarum Fabr. / det. A. Bertsch 1995'; (6) pale blue handwritten 'C01'; (7) green printed '*Bombus s. str.* / B# 194 det. PHW'; (8) red printed 'LECTOTYPE [female] / *Bombus lucorum* / *terrestricoloratus* / Krüger, 1951 / det. PH

Williams 2011'. This queen, which lacks part of the right mid leg and distal parts of the left fore and mid tarsi, is designated here as **LECTOTYPE** in order to reduce uncertainty in the identity and application of the name. Examined.

Barcode type: B#194 direct from the lectotype (Bertsch, 2009).

Unfortunately the original sequence is not yet publicly available (A. Bertsch, pers. com.). However, following the information given by Bertsch (2009), it is in principle possible to reconstruct at least part of the original barcode sequence from his published data by interpolating the 'informative triplets' for *terrestricoloratus* given in his fig. 2.1-4 from a reference sequence for a specimen identified as *B. cryptarum* from the same figure that has been published in GenBank, AY181124 (from Switzerland). This particular sequence is chosen because: (1) it is included in his fig. 2.1-4 with apparently all distinguishing nucleotides noted; (2) it clusters close to his sequence for *terrestricoloratus* in his fig. 4; and (3) from my morphological examination, *terrestricoloratus* appears at present most similar to *B. cryptarum*. The combined sequence is then cropped to span just those nucleotide positions published for *terrestricoloratus*. The reliability of this approach must remain very much in question. Voucher specimen in the ZMA.

***terrestriformis* (*terrestris*)**

[*Bombus*] *Tb.* [(*Terrestribombus*)] *lucorum* [var. geogr.] *terrestriformis* Vogt, 1911: 56.

Availability: Vogt's (1911: 50 footnote) statements show that he intended 'Varietas geographica' to be equivalent to subspecies in rank (ICZN, 1999: Art. 45.6.4).

Nomenclature: As far as I am aware, no replacement name exists for the taxon from Turkey that had been recognized previously under this name as a subspecies of *B. lucorum* (a misidentification, see below) by Rasmont (1984).

Type locality from original publication: 'Sultan Dag (Asia minor)' (Sultandagi, Turkey).

Type material: syntype queens (ZMA). Syntype queen (ZMA, 'SultanDag', labelled as lectotype by T. De Meulemeester and P. Rasmont, unpublished) examined. The syntypes of this taxon have been misidentified as being conspecific with *lucorum* (L.) but are in fact conspecific with *terrestris* (L.). This interpretation is shown on a label added by P. Rasmont and I agree with this.

Barcode proxy type: B#134 from a queen, from Sinop, Turkey (collected by F. Dikmen, in 2010). This matches the revised concept of this taxon in colour pattern and morphology. Specimen in the project voucher collection.

***terrestris* (*terrestris*)**

[*APIS*] *terreftris* Linnaeus, 1758: 578 no. 30.

Type locality from original publication: 'in Europæ terra' (Uppland, Sweden, according to Løken, 1973: 53).

Type material: neotype queen (NHR) from Uppland, Sweden, by designation of ICZN Opinion 1828 (1996: 64). Not seen, but taxon concept not in doubt.

Barcode proxy type: B#562 from a queen, from Predikstolen, Uppsala, Sweden (collected by B. Cederberg, in 2011). This

matches the usual concept of this taxon in colour pattern and morphology. Specimen in the project voucher collection.

***terricola* (*terricola*)**

BOMBUS TERRICOLA Kirby, 1837: 273.

Type locality from original publication: 'Lat. 65°' [North] (Canada).

Type material: holotype queen by monotypy, presumed lost (Franklin, 1913: 274; Milliron, 1971: 53). However, the identity of this species is not in doubt from the description and original illustration, so designation of a neotype is unjustified (ICZN, 1999: Art. 75.1).

Barcode proxy type: B#231 from a male, from 62.58° N in the Northwest Territories, Canada (collected by A. Gunn, in 2004). Voucher specimen in the LP collection.

***tunicatus* (*tunicatus*)**

Bombus tunicatus Smith, 1852: 43.

Type locality from original publication: 'Chusan' (Zhoushan, Zhejiang, China), apparently false as this species is restricted to the Himalaya (Williams, 1991).

Type material: lectotype queen (BMNH) from 'N. India' by designation of Tkalcu (1974a: 325). Examined.

Barcode proxy type: B#115 from a queen, from Gothichaur, Nepal (collected by F. Creutzburg, in 2007). This matches the type specimen in colour pattern and morphology. Specimen in the project voucher collection.

***turkestanicus* (?*cryptarum*)**

Bombus [(*Terrestribombus*)] *magnus*, Rasse *turkestanicus* Krüger, 1954: 274.

Availability: Krüger's (1951: 142) statements show that he intended 'geographische Rassen' to be equivalent to subspecies in rank (ICZN, 1999: Art. 45.6.4).

Nomenclature: junior primary homonym in *Bombus* of *Bombus mendax* var. *turkestanicus* Skorikov (1910: 329).

Type locality from original publication: 'Forts [sic] Narynj am gleichnamigen Flusse' (Naryn, Kyrgyzstan).

Type material: syntype queens, workers, males (ZMA). Syntype queen (ZMA, 'Narynj', labelled as lectotype by P. Rasmont, unpublished) examined.

Barcode proxy type: B#278 from a worker, from close to Naryn, Kyrgyzstan (collected by L. Best, in 2009). Because according to our results at least two morphologically closely similar species of *Bombus s. str.* occur in the broad region of the type locality (*B. cryptarum*, *B. lucorum*), associating sequenced specimens of either of these species with an unsequenced type specimen (*B. magnus turkestanicus*) has to be subjective and provisional. Available morphological diagnoses (Rasmont, 1984) are difficult and are unproven outside Europe, but appear consistent with this interpretation. The prospective lectotype shares with *B. cryptarum* (cf. *B. lucorum*): (1) clypeus with punctures in the medial ventral area denser, larger and deeper; (2) metasomal tergum 2 with punctures in the medial posterior

area denser with stronger intervening surface sculpturing. Specimen in the project voucher collection.

vasilievi (*lantschouensis*)

B.[ombus] vasilievi Skorikov, 1913: 172.

Type locality from original publication: ‘Charbin’ (Harbin, Heilongjiang, China), and ‘Gan-su’ (Gansu, China).

Type material: syntype queen (ZISP) examined, syntype queen (ZMA) examined. One queen (ZISP) agrees with the original description and carries the labels: (1) handwritten in Russian ‘[Harbin/Vasiliev]’; (2) white printed ‘K. Skorikov’; (3) red printed ‘*Lectotypus Bombus/vasilievi* Skorikov/design. Podbolotsk.’ (unpublished); (4) red printed ‘LECTOTYPE [female]/*Bombus/vasilievi*/Skorikov, 1913/det.

PH Williams 2011’; (5) white printed ‘[female] *Bombus/(Bombus)/lantschouensis*/det. PH Williams 2011’. This queen, which lacks a part of the right antenna, small parts of both forewings and part of the right hind tarsus, is designated here as **LECTOTYPE** in order to reduce uncertainty in the identity and application of the name. Examined.

Barcode proxy type: B#251 from a male, from Shuangjingdian, Neimenggu, China (collected by P. Williams, in 2007), the closest available sample. This matches the concept of this taxon in colour pattern and morphology. Because Shuangjingdian is not very close to Harbin and because *vasilievi* is closely similar to *lantschouensis* and not geographically discrete, only the suggested proxy is labelled *vasilievi*. Specimen in the project voucher collection.