

Bombus cullumanus—an extinct European bumblebee species?

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Abstract – *Bombus cullumanus s. str.* has attracted some of the greatest conservation concerns among bumblebees in Europe because it might now be extinct. However, there has been long-standing disagreement about whether it is conspecific with other eastern pale-banded bumblebees. We investigate these relationships using new data from DNA (COI) barcodes. The results support a Nearctic *rufocinctus*-group (*Bombus rufocinctus*) and a Palearctic *cullumanus*-group, the latter with just three species: *Bombus semenoviellus*, *Bombus unicus* and *B. cullumanus s. l.* (including several differently coloured taxa). We conclude that, although any persisting *B. cullumanus cullumanus s. str.* might be a regional conservation priority within Europe, nevertheless, because the species remains common elsewhere within its range in Asia, globally a higher conservation priority should be given to *B. unicus*, which is genetically more distinct and appears to have a much smaller population in the Russian Far East.

bumblebee / *Bombus* / barcode / taxonomy / conservation

1. INTRODUCTION

The species in the *cullumanus*-group include perhaps the most threatened bumblebees in Europe. However, there have been strongly differing interpretations of the number of species, so we urgently need to resolve this taxonomic confusion, particularly when seeking to formulate much-needed conservation measures.

Apis cullumana Kirby (1802) was first described from a yellow-banded male from Witnesham, in Suffolk, Britain (the holotype: Yarrow 1968). It was named after Sir Thomas Cullum, seventh Baronet, who was a local natural historian. But it was not until more than a century later that these males and the name

were associated in Britain with the conspecific unbanded, red-tailed females (Richards 1926). The species has always been rare in Britain, which, when compounded with the challenge of the close resemblance in colour pattern and morphology of the females to those of the widespread and abundant *Bombus lapidarius* (Linnaeus), has resulted in very few British females of *Bombus cullumanus* ever having been recognised (Yarrow 1954). The last record of the species in Britain was in ‘c. 1941’ (BMNH collection: Williams and Osborne, 2009), and it is widely believed that *B. cullumanus* has been extirpated there.

Taxonomic disagreements centre on the relationship between the British population of *B. cullumanus s. str.* and its close relatives elsewhere. Bumblebees with similar morphology and a dark unbanded female colour pattern of the pile have been recorded in a few places in Western

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Europe: in France, Belgium, Denmark, northwest Germany and Sweden (e.g. Nevinson 1923; Skorikov 1923; Richards 1926; Rasmont et al. 2005a), and these have been accepted universally as parts of the same species. However, several other similar taxa with female colour patterns with broad light yellow bands (*Bombus serrisquama* Morawitz from Kazakhstan), darker or reduced yellow bands (e.g. *Bombus silantjewi tenuifasciatus* Vogt [a junior homonym] and *Bombus silantjewi nigrotaeniatus* Vogt from Kazakhstan, *Bombus popovi* Panfilov from Tajikistan, and *Bombus praemarinus* Panfilov possibly from Primorsky), or broad white bands (*Bombus apollineus* Skorikov from Armenia) have been described from across the Palaearctic region (Vogt 1911; Panfilov 1951: mapped in his Figure 7). The type material for *B. popovi* cannot be found at present, so we cannot be sure of its identity. Closely similar to *B. silantjewi nigrotaeniatus*, *B. praemarinus* (Panfilov 1951) has reduced yellow bands on the thorax and very little yellow pile on the metasoma. It was described from only two Russian specimens: the holotype female, collected in 1903 from Vladivostok, Primorsky (Institute of Zoology, St Petersburg, PW examined) and another female described in a note added in press, collected in 1947 from Mondy, Buryatia (Zoological Museum, Moscow State University, AB examined). No further individuals from near the published type locality in Primorsky have been recorded since (M. Proshchalykin, in lit.). These bees have a dark colour pattern with very little yellow pile on the scutellum and metasoma. Although according to the description, they have especially dense punctures on the clypeus and temples, the differences do not appear to be large. However, the precise size and pattern of these punctures varies among all of these bees, although this has not yet been assessed quantitatively.

The taxa were first revised as a group in detail by Panfilov (1951), who interpreted them as separate species (a view shared by Skorikov 1923; Yarrow 1954), arguing that this is evidenced by small differences in morphology.

Rasmont (1988), reviewing the fauna of France, commented on how difficult it was to draw conclusions for such rare taxa with very few specimens, but believed that the colour-based taxa are likely to be separate species because there was no evidence of intermediates from the few regions where they occurred in close proximity that had been sampled (e.g. Pyrenees, Elburz), and because they appeared to occupy slightly different habitats. Williams (1998), reviewing the world fauna, interpreted several of these taxa as parts of a single broadly distributed Palaearctic species (a view shared earlier by Kruseman 1959; Tkalcu 1969; Reinig 1971; Løken 1973; Ornos 1986), arguing that there were no clearly distinct morphological character states other than colour from which to diagnose them. The distinctive male labial gland secretions of *B. cullumanus* and *Bombus semenoviellus* have been characterized by Hovorka et al. (2006), and similar studies of other taxa in this group are in progress.

DNA analysis has recently added a potential source of large numbers of new characters for informing these taxonomic questions as applied to bees (Gibbs 2009; Packer et al. 2009). Several different genes have been used successfully in phylogenetic studies of bumblebees, although for particularly closely related taxa (such as within the *cullumanus*-group), the most information-rich sources are the fast-evolving mitochondrial genes (Cameron et al. 2007), which include COI. Even very short sequences of COI can diagnose the majority of species tested by Meusnier et al. (2008), making COI barcoding a cost-effective approach to this kind of problem, especially as it can often be applied easily to pinned specimens if they are less than 20 years old. Barcoding bumblebees of the subgenus *Subterraneobombus* worldwide has demonstrated that COI can help to diagnose conventional morphological species while, in that case, at least not resulting in recognising many apparently new but artifactual 'cryptic species' (Williams et al. 2011). Therefore, in this study, we use COI barcoding of recent museum specimens to explore whether DNA data can provide evidence to illuminate the

question of the status of taxa within the *cullumanus*-group. In particular, we ask what the taxonomic limits of the species *B. cullumanus* are and what the consequences are of that for conservation planning?

2. METHODS

2.1. Material

Bombus eximius Smith and *Bombus sibiricus* (Fabricius) are used here to represent outgroups to the *cullumanus*-group based on the estimate of phylogeny for nearly all bumblebee species obtained from five genes by Cameron et al. (2007). Following the same phylogenetic estimate, we include *Bombus morrisoni* Cresson and *Bombus crotchii* Cresson to represent the rest of the subgenus *Cullumanobombus* in the broad sense of Williams et al. (2008). Samples were obtained from 49 individuals showing differing morphology and colour pattern of the pile and representing almost all of the taxa that have been regarded as separate species within Richards' (1968) narrower concept of the subgenus *Cullumanobombus* (Table 1). In the Old World, these bees are often rare, and few recent samples are available from collections. We are able to include a sample from one of the last known specimens (the only known recent specimen) of *B. cullumanus* s. str., from France. Five specimens of *B. serrisquama* from Spain were sent for sequencing, but no sequences could be obtained

2.2. DNA barcoding

DNA was sequenced for the short 5' barcode region of the mitochondrial-encoded COI gene (cytochrome *c* oxidase, subunit 1). The sequenced samples were taken from specimens collected mostly within the last 15 years. Specimens were processed 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). DNA extraction, amplification and sequencing used the standard protocols described by Hebert et al. (2003). Universal primers for amplifying the COI-barcode sequence for insects were used (variants

LepF1 and *LepR1*; Hebert et al. 2004). No amplification of *Wolbachia* (or other obviously non-target DNA) was detected. Data for the specimens processed at Guelph have been uploaded to the BOLD online database (boldsystems.org; Ratnasingham and Hebert, 2007) and GenBank.

2.3. Reconstructing phylogeny

COI-barcode sequences (without primer sequences) from 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 was shared by most samples. We used MrBayes (version 3.1.2; Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) to search for estimates of the phylogenetic tree, from ten million generations of the metropolis-coupled Markov-chain Monte Carlo algorithm with four chains, chain temperature set to 0.5 and with sampling of the trees every 1,000 generations. We found the nucleotide-substitution model that fitted our COI-barcode data best according to jModelTest (version 0.1.1; Posada 2008) to be the general time-reversible model with an inverse-gamma frequency distribution of changes among sites. Burn-in was set to 10 % of the generations, with convergence between two separate runs of the analysis judged to have occurred when the average standard deviation of the split frequencies approached stationarity. Post burn-in stability of the log likelihood of the cold chain was confirmed using Tracer (version 1.5.0; Drummond and Rambaut 2007), and stability of the sample groups was confirmed using AWTY (version 0.8.1; Nylander et al. 2008). Post burn-in sample trees from both replicates were combined, and trees were rooted using data for *B. eximius* to represent an outgroup, based on the results of Cameron et al. (2007).

2.4. Recognising species

There is no universally accepted concept of the nature of species, or any universally accepted method for their recognition in practice (Mallet 1995, 1997). All of the concepts have their counter arguments. One possibility that has been discussed is the idea of a

Table 1. Material examined with BOLD COI sequence numbers

| Taxon | Caste | Locality | Collector | BOLD specimen number | In-frame sequence length (bp) | Depository |
|-----------------------|-------|-------------------------------|--------------|----------------------|-------------------------------|-------------|
| <i>apollineus</i> | w | Armenia, Sevan | S. Droege | 1549E08 | 594 | P. Williams |
| <i>apollineus</i> | m | Turkey, Erzurum | P. Rasmont | 1551H03 | 612 | P. Rasmont |
| <i>apollineus</i> | m | Turkey, Kars | P. Rasmont | 6879D09 | 658 | P. Rasmont |
| <i>apollineus</i> | m | Turkey, Kars | P. Rasmont | 6879D10 | 658 | P. Rasmont |
| <i>apollineus</i> | m | Turkey, Kars | P. Rasmont | 6879D11 | 658 | P. Rasmont |
| <i>apollineus</i> | m | Turkey, Kars | P. Rasmont | 6879D12 | 658 | P. Rasmont |
| <i>crotchii</i> | w | USA, California | S. Droege | 08-CA-2051 | 644 | L. Packer |
| <i>cullumanus</i> | m | France, Eyne | P. Rasmont | 1551H02 | 622 | P. Rasmont |
| <i>eximius</i> | q | Thailand, Tat Tone NP | Y. Areeluck | 3261H06 | 624 | L. Packer |
| <i>morrisoni</i> | q | USA, Utah | Andrus | 01-UT-2597 | 658 | L. Packer |
| <i>nigrotaeniatus</i> | w | Kyrgyzstan, Ak-Bulak | L. Best | 1551D10 | 622 | P. Williams |
| <i>nigrotaeniatus</i> | w | Kyrgyzstan, Alekseyevka | L. Best | 1551D11 | 425 | P. Williams |
| <i>nigrotaeniatus</i> | q | Kyrgyzstan, Krasnyj Oktyabr* | L. Best | 1551D08 | 658 | P. Williams |
| <i>rufocinctus</i> | q | Canada, Alberta | L. Morandin | 03-BC-0329 | 656 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Alberta | L. Morandin | 03-BC-0330 | 656 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Alberta | L. Morandin | 03-BC-0331 | 656 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Alberta | L. Morandin | 03-BC-0332 | 656 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Alberta | L. Morandin | 03-BC-0333 | 656 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Alberta | L. Morandin | 03-BC-0334 | 656 | L. Packer |
| <i>rufocinctus</i> | q | Canada, New Brunswick | C. Sheffield | sheffc80 | 403 | L. Packer |
| <i>rufocinctus</i> | q | Canada, New Brunswick | C. Sheffield | sheffc81 | 393 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Newfoundland | C. Sheffield | 09-NL-3037 | 658 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Northwest Territories | L. Morandin | 05-NT-0041 | 658 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Nova Scotia | C. Sheffield | Bee166 | 626 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Nova Scotia | C. Sheffield | sheffc79 | 609 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Ontario | S. Colla | 3742E06 | 658 | L. Packer |
| <i>rufocinctus</i> | q | Canada, Ontario | L. Packer | 0289G06 | 658 | L. Packer |

Table 1. (continued)

| Taxon | Caste | Locality | Collector | BOLD specimen number | In-frame sequence length (bp) | Depository |
|----------------------|-------|-------------------------------|---------------------------|----------------------|-------------------------------|-----------------------|
| <i>rufocinctus</i> | | USA, Colorado | B. and J. Thompson | 3760E09 | 565 | L. Packer |
| <i>rufocinctus</i> | | USA, Colorado | B. and J. Thompson | 3760E10 | 549 | L. Packer |
| <i>rufocinctus</i> | q | USA, Idaho | J. Gibbs and C. Sheffield | 07-ID-1427 | 658 | L. Packer |
| <i>rufocinctus</i> | q | USA, Washington | J. Gibbs and C. Sheffield | 07-WA-1428 | 658 | L. Packer |
| <i>semenoviellus</i> | w | Czech Republic, Pleše | L. Dvořák | 1549H09 | 658 | P. Williams |
| <i>semenoviellus</i> | m | Czech Republic, Plsečný-vršek | J. Straka | 1549H11 | 658 | P. Williams |
| <i>semenoviellus</i> | m | Germany, Brandenburg | C. Saure | 1549H10 | 626 | P. Williams |
| <i>semenoviellus</i> | q | Latvia, Keizari | M. Kalniņš | 6878A01 | 658 | P. Williams |
| <i>semenoviellus</i> | q | Latvia, Keizari | M. Kalniņš | 6878A02 | 658 | P. Williams |
| <i>semenoviellus</i> | q | Russia, Novosibirsk | Y. Yurchenko | 1549E01 | 653 | P. Williams |
| <i>semenoviellus</i> | q | Russia, Novosibirsk | Y. Yurchenko | 1549E02 | 583 | P. Williams |
| <i>serrisquama</i> | w | Kyrgyzstan, Ak-Bulak | L. Best | 1551D09 | 658 | P. Williams |
| <i>serrisquama</i> | q | Russia, Altai Territory | A. Byvalyev | 1549E06 | 658 | P. Williams |
| <i>serrisquama</i> | w | Russia, Irkutsk | D. Michez | 6878F05 | 658 | P. Williams |
| <i>serrisquama</i> | q | Russia, Moscow | T. Levchenko | PCHELA-C03 | 658 | Moscow University |
| <i>serrisquama</i> | m | Russia, Novosibirsk | A. Byvalyev | 1549E03 | 658 | P. Williams |
| <i>serrisquama</i> | m | Russia, Novosibirsk | A. Byvalyev | 1549E04 | 658 | P. Williams |
| <i>serrisquama</i> | m | Turkey, Kars | P. Rasmont | 6879E01 | 573 | P. Rasmont |
| <i>serrisquama</i> | m | Turkey, Kars | P. Rasmont | 6879E02 | 632 | P. Rasmont |
| <i>serrisquama</i> | m | Turkey, Kars | P. Rasmont | 6879E04 | 658 | P. Rasmont |
| <i>sibiricus</i> | w | Mongolia, Zavkhan | J. Gelhaus | 1550G03 | 658 | University of Kansas |
| <i>tenuiasciatus</i> | w | Kyrgyzstan, Alekseyevka | L. Best | 1551D12 | 425 | P. Williams |
| <i>tenuiasciatus</i> | q | Kyrgyzstan, Krasnyi Oktyabr' | L. Best | 1551D07 | 658 | P. Williams |
| <i>unicus</i> | q | Russia, Amur | M. Proshalykin | 6875A07 | 658 | Vladivostok Institute |
| <i>unicus</i> | q | Russia, Amur | M. Proshalykin | 6875A08 | 658 | Vladivostok Institute |
| <i>unicus</i> | q | Russia, Amur | M. Proshalykin | 6875A09 | 658 | Vladivostok Institute |

Caste abbreviations: q, queen; w, worker; m, male

barcoding ‘gap’ between the relative amounts of COI-sequence divergence expected between and within species. This approach has been much criticised when reduced to a single generalised divergence-threshold criterion (Meyer and Paulay 2005; Meier et al., 2006; Schmidt and Sperling 2008).

Although a fixed numerical criterion for recognising species from the amount of divergence may be unreliable, a more flexible but related approach, focussing instead on changes in the branching pattern near the species level (between interspecific and intraspecific regions of the tree), has also been discussed (e.g. Monaghan et al. 2005; Papadopoulou et al. 2008; Pons et al. 2006). Unfortunately, fully quantitative models of these processes are only applicable in situations where many species are anticipated with multiple unique haplotypes within each (Monaghan et al. 2009), which is not the case here. Nonetheless, the principle remains applicable by inspection of the resulting tree and distances. MEGA (version 4.0; Tamura et al. 2007) was used to measure intra- and inter-group sequence divergences, which were calculated using the Kimura two-parameter distance model (Kimura 1980).

3. RESULTS

Figure 1 shows that all *Bombus rufocinctus* samples are supported as a group and that this is the sister group to a group including the *cullumanus*-group (*apollineus*+*serrisquama*+*tenuifasciatus*+*nigrotaeniatus*+*cullumanus* s. str.) plus a large New World group (including *morrisoni*+*crotchii*).

COI differentiation within the *rufocinctus*-group is generally weak, despite the large geographic range encompassed, with only the New Brunswick samples showing a distinct group. Evidence to support the monophyletic group *apollineus*+*serrisquama*+*tenuifasciatus*+*nigrotaeniatus*+*cullumanus* s. str. is strong (Figure 1). From both the long branches between the groups interpreted as species supported by the many uniquely shared diagnostic polymorphisms (minima of sequence divergence ≥ 6.15 %, Table II) and the distinctly shorter branches within the groups (maxima of

sequence divergence ≤ 2.4 %, Table II), we conclude that there is no evidence from the COI-barcode analysis (Figure 1) for recognising more than the following species: *B. rufocinctus*, *B. semenoviellus*, *Bombus unicus*, and the single broadly distributed species *B. cullumanus* s. l. The mean intraspecific divergence within *B. cullumanus* s. l. is especially small given the number of sequences and the breadth of geographic coverage in the sample (Table II).

4. DISCUSSION

There are many potential pitfalls in the interpretation of COI barcodes in terms of the evolution of taxa (Rubinoff et al. 2006). Those most likely to confound our analysis are that patterns of evolution for mitochondrial genes do not always correspond precisely with those of their owner species. However, these problems have not yet been detected in studies of bumblebees (Bertsch 2010; Williams et al. 2011).

Our tree (Figure 1) shows a strong pattern of a distinct separation between many short terminal branches and a few long higher branches (with many diagnostic nucleotide changes, Table II). Elsewhere, this kind of pattern has been interpreted as marking the separation between small intraspecific differences and larger interspecific differences (Monaghan et al. 2005, 2009; Pons et al. 2006; Papadopoulou et al. 2008). Such a strong distinction (corresponding to the much criticised ‘barcoding gap’) is not universal among bumblebees (e.g. Williams et al. 2011) but is nonetheless apparent in this case. Nonetheless, it would be desirable to compare our COI results for these bees with results from a highly variable nuclear gene.

Our results agree with Franklin (1913), Frison (1927), Milliron (1973) and Plowright and Owen (1980) in supporting just one variable species *B. rufocinctus* in North America. This has long been recognised to be the most variable species in colour pattern within the entire western hemisphere (Franklin 1913). Geographic structure within this variation is

Table II. Divergence in COI-barcode sequences among and within inferred species

| Species | Number of sequences | Number of uniquely shared diagnostic polymorphisms (non-synonymous) | Minimum interspecific sequence divergence (%) | Maximum intraspecific sequence divergence (%) | Mean intraspecific sequence divergence (%) |
|----------------------------|---------------------|---------------------------------------------------------------------|-----------------------------------------------|-----------------------------------------------|--------------------------------------------|
| <i>B. rufocinctus</i> | 18 | 28 (28) | 9.64 | 2.40 | 0.76 |
| <i>B. semenoviellus</i> | 7 | 13 (12) | 6.15 | 0.69 | 0.31 |
| <i>B. unicus</i> | 3 | 10 (10) | 6.15 | 0.00 | 0.00 |
| <i>B. cullumanus s. l.</i> | 21 | 22 (22) | 6.73 | 0.48 | 0.09 |

Sequence divergences are calculated using the Kimura two-parameter (K2P) distance model

colour pattern. The Turkish and Armenian samples (the southern part of the global range) all have broad pale bands, although the bands may be either white or yellow, without intermediates. In this particular region, the white-banded female colour pattern (*apollineus*: see map in Panfilov 1951) is a frequent convergent colour pattern (Williams 2007), and other species show a similar yellow/white dimorphism (Williams 1998; Rasmont et al. 2005b; De Meulemeester et al. 2010). The remaining more northern samples either have broad yellow bands or reduced yellow bands. In the Russian steppe and sometimes in Central Asia, females have broad bands (*serrisquama*: see map in Panfilov 1951). This is the most common colour pattern among all bumblebees worldwide, and extensively yellow patterns are especially frequent among steppe bumblebees (Williams 2007). Darker female colour patterns also occur in Central Asia, some with darker yellow bands (e.g. *tenuifasciatus*) and some with very little yellow on the metasoma (e.g. *nigrotaeniatus*, probably including at least the second female of *praemarinus*, described from Buryatia, although it is just possible that the holotype of *praemarinus* is not actually from Vladivostok but might also have originated from Central Asia). The darkest female colour pattern, lacking all of the yellow bands (*cullumanus s. str.*), is confined to southern England and adjacent parts of north-western continental Europe from the Pyrenees to southern Sweden (Rasmont et al. 2005a). In this region, the dark colour

pattern is especially frequent among bumblebee species (Williams 2007).

The conservation status of the *cullumanus*-group in Europe has been a subject of concern for many years. Individuals with the dark female colour pattern, *B. cullumanus cullumanus s. str.*, are known to be in strong decline in Britain (Yarrow 1954; Alford 1975), in France (Rasmont 1988; Rasmont et al. 2005a), and in Western Europe generally (Kosior et al. 2007). A remnant population with this colour pattern was known in the French Pyrenees (Iserbyt et al. 2008) at least as recently as 2001, but the very last live individual with this colour pattern to be seen was in the Massif Central of France in 2004. The light yellow female colour pattern, *B. cullumanus serrisquama*, is in decline in Hungary (Sárospataki et al. 2005) and in Western Europe generally (Kosior et al. 2007), where it is now very rare. However, in the steppes of the west Siberian plain, it is still the dominant or subdominant species of bumblebee ($\geq 10\%$ of the total number of bumblebees) in 13 of the 36 localities sampled by one of us (Table III). It is also still common in some collections from China (Xinjiang), Kazakhstan and Kyrgyzstan (Williams 2011), as are the darker *B. cullumanus tenuifasciatus* and *B. cullumanus nigrotaeniatus* in the same region. Individuals with the white-banded colour pattern, *B. cullumanus apollineus*, were once very common in Turkey, but they have become rarer, although still present (M. Aytekin, in lit.). One of us (PR) found abundant and apparently stable populations of *B. cullumanus apollineus* in the

Table III. Numbers of bumblebees including *B. cullumanus* counted in Siberia (AB) based on the method of Pesenko (1972) using a sweep-net along transects of 2×100 m searched for 20 min and expressed as the number of individuals per hour

| Site name | GPS coordinates | Date | Total no. of <i>Bombus</i> individuals per hour | No. of <i>Bombus</i> spp. | No. of <i>B. cullumanus</i> individuals per hour | Percentage of <i>B. cullumanus</i> individuals |
|----------------|-----------------|--------|-------------------------------------------------|---------------------------|--------------------------------------------------|------------------------------------------------|
| Siberia | | | | | | |
| Mikchailovskoe | 51°49'N 79°36'E | 7/2005 | 3.0±3.0 | 1 | 3.0±3.0 | 100.0 |
| Melnikovo | 52°14'N 81°06'E | 7/2006 | 36.0±3.0 | 3 | 18.0±3.0 | 50.0 |
| Kamen-na-Obi | 53°44'N 81°07'E | 7/2007 | 12.0±3.0 | 2 | 4.5±1.5 | 37.5 |
| Oktyabrskoe | 55°00'N 67°54'E | 6/2005 | 17.4±2.7 | 14 | 5.3±3.2 | 30.5 |
| Troitskoe | 53°42'N 77°49'E | 8/2006 | 40.5±6.1 | 16 | 11.5±3.5 | 28.4 |
| Mikchailovskoe | 51°49'N 79°36'E | 7/2007 | 18±3.0 | 2 | 4.5±1.5 | 25.0 |
| Klepechiha | 52°05'N 81°44'E | 7/2006 | 4.5±1.0 | 5 | 1.0±0.9 | 22.2 |
| Klepechiha | 52°05'N 81°44'E | 7/2008 | 30.8±5.7 | 7 | 6.8±3.3 | 22.1 |
| Novonikolskoe | 54°32'N 68°37'E | 7/2005 | 47.0±4.4 | 12 | 10.0±5.6 | 21.3 |
| Grachevo | 55°29'N 65°41'E | 6/2005 | 21.7±5.4 | 15 | 4.5±1.8 | 20.7 |
| Pokrovka | 52°02'N 79°20'E | 7/2007 | 24.±6.0 | 4 | 4.5±1.5 | 18.7 |
| Zolotoe | 55°06'N 66°56'E | 6/2005 | 16.3±2.2 | 18 | 2.8±0.7 | 17.2 |
| Bor-Forpost | 51°52'N 80°06'E | 7/2008 | 9.0±0.0 | 2 | 1.5±1.5 | 16.7 |
| Kochki | 54°18'N 80°25'E | 8/2007 | 70.5±8.1 | 16 | 10.0±3.0 | 14.2 |
| Alexandrovskii | 53°39'N 78°15'E | 7/2007 | 11.5±3.2 | 10 | 1.5±1.5 | 13.0 |
| Alexandrovskii | 53°39'N 78°15'E | 7/2008 | 29.5±5.4 | 13 | 3.5±1.4 | 11.9 |
| Petropavlovsk | 54°50'N 69°03'E | 8/2006 | 51.5±4.3 | 8 | 6.0±2.9 | 11.7 |
| Turkey | | | | | | |
| Kars, Göldalı | 40°58'N 43°18'E | 8/2011 | 79 | 9 | 62 | 78.4 |

The date refers to the month and year of the recording period. The last line is a sample from northeastern Turkey (PR and A. M. Aytekin)

Kars region, where it is intermixed with scattered *B. cullumanus serrisquama*.

B. semenoviellus is another species that is rare in collections. Against the general declining trend among many European bumblebee species, it has expanded its range westwards recently, as evidenced by several recent papers (van der Smitsen and Rasmont 1999; Přidal and Tkalců 2003; Přidal and Komzáková 2009; Streinzer 2010). *B. unicus* is known from very few specimens from the Russian Far East, although no targeted surveys have been made (M. Proshchalykin, in lit.).

All of the Old World *cullumanus*-group species are very rare or potentially threat-

ened, at least in some parts of their ranges. The dark individuals of *B. cullumanus cullumanus* s. str. may now be extinct, but according to our results, other closely related individuals of *B. cullumanus serrisquama* and *B. cullumanus apollineus* appear to be relatively unthreatened. Therefore, although the apparent loss of individuals with this dark colour pattern from Western Europe (and any special adaptive genes it might have had) is very unfortunate; nonetheless, the positive view is that at least our tree would appear to predict that the amount of unique diversity lost in all other genes (assuming that the COI tree is predictive because of shared inheri-

tance) is less than might have been the case if it had been more distantly related to the eastern part of the population (Vane-Wright et al. 1991). It would be useful to know whether any special characteristics of the dark western *B. cullumanus cullumanus s. str.* are also shared by the dark individuals of *B. cullumanus (tenuifasciatus, nigrotaeniatus)* from Central Asia.

What are the consequences of our results for conservation? In a European context, *B. cullumanus cullumanus s. str.*, if it still exists anywhere, would be one of the highest priorities. But in a global context, our tree would identify the far eastern *B. unicus* as an even higher priority for conservation action, because of its greater unique genetic divergence, combined with the restricted geographic range and low abundance of the entire species. We urgently need surveys to document the status of *B. unicus*, the principal threats and its vulnerability.

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***Bombus cullumanus*—une espèce de bourdon européen éteinte?**

***Bombus* / bourdon / Europe / taxonomie / conservation des espèces / code barre génétique**

***Bombus cullumanus*—eine ausgestorbene europäische Hummelart?**

Hummeln / *Bombus* / Barcode / Taxonomie / Naturschutz

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