DISCOVERY OF *BOMBUS DISTINGUENDUS* (HYMENOPTERA: APIDAE) IN CONTINENTAL NORTH AMERICA[1]

C. S. SHEFFIELD[2] AND P. H. WILLIAMS[3]

Department of Biology, York University,
4700 Keele Street, Toronto, ON, Canada M3J 1P3
email: cory.silas.sheffield@gmail.com

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The bumblebees of North America have received much attention, not only because these charismatic bees are important for pollination of native plants, but also because several bumblebee species have recently declined rapidly (Colla and Packer 2008; Grixti et al. 2009; Williams and Osborne 2009; Cameron et al. 2011). As a result, the North American fauna is one of the best known (Williams 1998). However, even for such a well-studied group, the taxonomic status of several species in North America remains unclear because of unique and geographically separate colour forms with very few specimens (e.g., *B. cockerelli* Franklin), close affinities with Old World species complexes (e.g., *B. moderatus* Cresson; Scholl et al. 1990), and variable intra- and interspecific colour patterns (e.g., Stephen 1957; Williams 2007; Owen et al. 2010). These difficult cases have prompted the application of molecular methods (e.g., DNA barcoding) to supplement traditional morphology-based taxonomic study (Murray et al. 2008; Bertsch et al. 2010; Owen et al. 2010; Williams et al. 2011).

Williams and Thomas (2005) recorded *B. distinguendus* Morawitz for the first time in the New World from Attu Island, at the far western end of the Aleutian archipelago. This discovery made *B. distinguendus* one of perhaps eight bumblebee species with a Holarctic distribution, though restricted to the western edge of North America. As part of an ongoing campaign to obtain COI sequences for the bees of the world, bumblebees from across the continent have been collected and/or donated by collaborators in Canada and the United States. In one series of specimens from Alaska, three females (two from Fairbanks, 64.747°N 148.086°W, 28.vii.2009 and 64.86°N 147.86°W, 11.vi.2009; one from Palmer, 61.567°N, 149.233°W, 18.v.2009), deposited in the Department of Biology, York University, Toronto, Canada, and The Natural History Museum, London, UK, were identified initially (by CSS) as *B. appositus* Cresson based on external morphology. These were then DNA barcoded (see Sheffield et al. 2009 for procedures) because Alaska would represent a northern range extension for this species (Stephen 1957; Milliron 1973), and sequences and images were loaded to the BOLD (Barcodes of Life Data System; http://www.boldsystems.org) library.

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[1] This paper is contribution #30 from the Canadian Pollination Initiative.

[2] Author to whom all correspondence should be addressed.

[3] Natural History Museum, Cromwell Road, London, UK SW7 5BD

Surprisingly, the sequences were unique among North American *Bombus* species, showing 1.55% divergence from the nearest neighbour, *B. appositus*, and matching those of *B. distinguendus* from Attu Island. An additional specimen, labelled "90106/Airport Willow Bar/ Fbnks Intl Airport/On Hedysarum boreale/25 May 90/J.A. Bishop", deposited in the University of Alaska Museum, Fairbanks, Alaska, and identified as *B. appositus*, was also examined. Identification of these specimens was later verified (by PHW) as *B. distinguendus*. Williams and Thomas (2005) and Williams et al. (2011) provided keys to separate the species; the latter give additional illustrations and diagnoses to separate the species. Further information on the specimens studied here, including COI sequence information (accession numbers, etc.), can be found in Williams et al. (2011).

In light of the discovery (Williams and Thomas 2005) and subsequent DNA barcoding of *B. distinguendus* from Attu Island, and with the DNA barcode-assisted discovery on continental North America reported here, the utility of DNA barcoding for detecting bees with previously unrecorded Holarctic distributions seems promising. However, the relationship among North American *B.* (*Subterraneobombus* Vogt) and the presence of *B. distinguendus* in continental North America is somewhat puzzling on the basis of COI results. Hines (2008) reported that vicariance events between the Old and New Worlds across Beringia involved splits among boreal species, including *B. appositus* and *B. borealis* from *B. distinguendus*. Supporting this, levels of COI divergence between North American *B. distinguendus* and the other *B.* (*Subterraneobombus*) are very low; 1.55% between *B. distinguendus* and *B. appositus*, and 1.86% between *B. distinguendus* and *B. borealis* Kirby, (Williams et al. 2011), probably attributable to the spread and recent (< 2 Myr) arrival of an ancestral *distinguendus* complex in the Nearctic (Williams 1985; Hines 2008).

Surprisingly, the Alaskan specimens (Attu Island and mainland) show greater COI sequence similarity to populations that are most geographically distant from them (Williams et al. 2011), namely, 0.3 ± 0.34% (max. 0.62%) sequence divergence between Alaska and UK, 0.93 ± 0.15% (max. 1.8%) between Alaska and Europe (excluding UK), and 1.1 ± 0.1% (max. 2.2%) between Alaska and the Russian Far East. These differences were reflected in the high level of divergence in this species across its range (2.67% maximum sequence divergence), which Williams et al. (2011) attribute to perhaps higher levels of habitat fragmentation and population isolation in the northern parts of its range during glacial cold periods.

Although some species of *Bombus* have been introduced to areas outside of their natural range, it seems unlikely that populations of *B. distinguendus* would have been deliberately introduced into southern Alaska, especially from the UK. Explanation of the similarity of COI between Alaska and UK populations is further confounded because the sequences conflict with the pattern of variation in pubescence colour. In this respect, the North American specimens actually resemble more closely the Old World populations that are geographically closer, in Russia (Williams et al. 2011), as would be expected (Hines 2008). Although it is tempting to suggest a possible thermoregulatory role for darker pubescence (Pekkarinen 1979), the principal global pattern is for darker forms in *Bombus* to be associated more with tropical climates (Williams 2007). However, the relationship between bumblebee colour pattern and thermoregulation is not well understood.

Although it seems clear that *B. distinguendus* was not introduced into North America by human activity, it is surprising that populations would have gone undetected for so long, as bumblebees have been one of the most intensively studied and heavily surveyed bee groups on this continent (Williams 1998). This species may simply be very rare in North America; it is presently only known from these three specimens reported above and the 17 specimens reported by Williams and Thomas (2005), and males have yet to be collected in North America. But it may also have been easily confused with *B. appositus* and *B. borealis*, less so with *B.* (*Thoracobombus*) *fervidus* (Fabricius), though only *B. appositus* and *B. borealis* have ranges that approach or include southern Alaska. Clearly, further studies incorporating traditional morphological and additional genetic (e.g., Schmid-Hempel et al. 2007; Lye et al. 2011) approaches for *Bombus* distribution and phylogeny are needed, and these may help resolve the puzzling COI sequence and colour form distributional patterns of *B. distinguendus*. The recent discovery of *B. distinguendus* highlights the need for more complete surveys of bees, especially in previously unsampled or poorly sampled areas, and for continued taxonomic study of these important pollinators.

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