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Scientific Note

A scientific note on range expansion of a sedentary bumble bee (*Bombus hortorum*) in New Zealand

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***Bombus hortorum* / *Bombus ruderatus* / bumble bee / molecular ecology**

Bombus hortorum is a long-tongued bumble bee that was introduced to New Zealand in 1906 to assist with red clover pollination (Hopkins 1914; Gurr 1964). Populations were successfully established in Canterbury (43.6° S 172.0° E) in the South Island. There appears to have been very limited subsequent dispersal until targeted northward translocations starting in 1965 (Gurr 1972). This contrasts with two other species of introduced bumble bee, *B. terrestris* and *B. ruderatus*, which had similarly limited ranges of introduction but are now widespread throughout New Zealand's main islands (Macfarlane and Gurr 1995) through natural dispersal. The relatively sedentary nature of *B. hortorum* is consistent with observations in the endemic range of this bumble bee where populations are common, but highly structured (Goulson et al. 2011).

With increasing interest in bumble bees as pollinators in commercial ecosystems (Velthuis and Van Doorn 2006; Howlett and Donovan 2010), we aimed to reassess the distribution of *B. hortorum* in New Zealand. One hundred and ninety-one long-tongued bumble bee samples were submitted to The New Zealand Institute for Plant & Food Research Ltd. in summer/autumn 2014, following a public request issued through the media and entomological networks. To differentiate *B. hortorum* from *B. ruderatus* (a morphologically indistinguishable, sympatric bumble bee), the HotSHOT (Truett et al. 2000) protocol was used to extract DNA from bee leg muscle and a restriction endonuclease digest of cytochrome *b* (Ellis et al. 2006) used to assign species (Figure 1). Negative and positive controls for all assays were included during analysis. Analysis was repeated on 10% of samples to assess reproducibility with complete congruence between datasets. The target amplicon was also sequenced in selected samples to confirm sequence associations with respective RFLP patterns. Of the samples submitted 49 were assigned as *B. hortorum*, 101 were assigned as *B. ruderatus*, and 41 failed to amplify.

In our study, we obtained *B. hortorum* bee samples from Hamilton (Figure 2; 37° 47′ S 175 ° 17′ E) which is over 350 km further north than the previously reported northern limit of this bumble bee species in Palmerston North (40°

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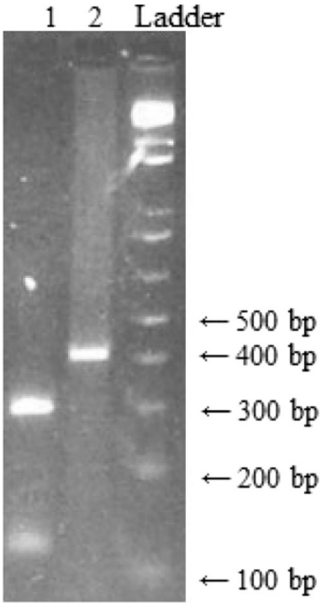

Figure 1. Image of 3% agarose gel showing the RFLP banding patterns used to distinguish *B. hortorum* and *B. ruderatus*. Following the protocol of Ellis et al. (2006), a target cytochrome *b* amplicon of *B. hortorum* (lane 1) contains a Tsp45I restriction endonuclease site and is digested into two fragments, 306 bp and 125 bp. Alternatively, the target cytochrome *b* amplicon of *B. ruderatus* (lane 2) does not contain a Tsp45I restriction endonuclease site and remains as a single fragment at 426 bp.

21.3′ S 175° 36.7′ E; Figure 2; Macfarlane and Gurr 1995). This range expansion has occurred over a period of less than 25 years and is particularly impressive for a normally sedentary species. An undocumented human-assisted translocation of *B. hortorum* may explain our observation, although there is no clear rationale for such an effort, as the morphologically indistinguishable bumble bee *B. ruderatus* already occurs in the expanded range. This observation highlights the risk of using species-specific historic range limits to differentiate between morphologically cryptic species, as *B. ruderatus* was formerly the only long-tongued bumble bee in the northern part of New Zealand, but will now require genetic delineation for future ecological studies.

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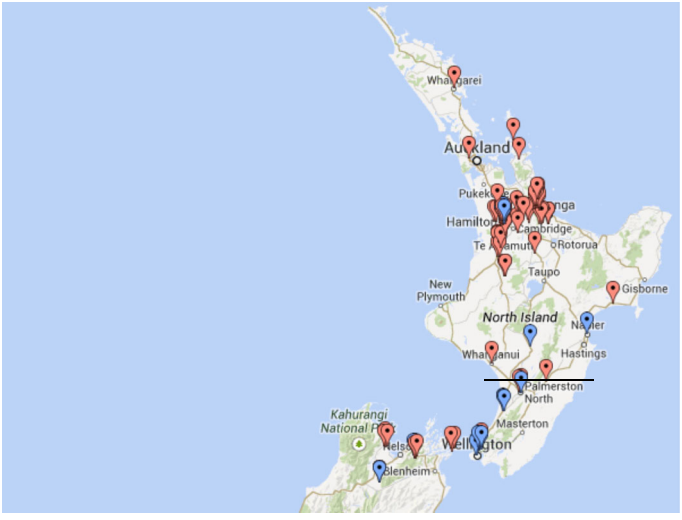

Figure 2. Map of New Zealand showing the catch locations of *Bombus hortorum* (blue tags) and *B. ruderatus* (red tags) in this study. The black horizontal line indicates the previously recorded northern limit of *B. hortorum*, as identified by Macfarlane and Gurr (1995). Map generated using http://batchgeo.com/ and Google maps.

AUTHORS' CONTRIBUTIONS

K. A.: Project planning. Data collection. Draft development; J.S.: Project design. Assay development. Data collection. Draft development; H.B.: Project design. Sample collection and curation. Editorial support; A.R.: Project design. Scientific oversight. Editorial support; D.P.: Project development and funding. Scientific oversight. Editorial support.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest The authors declare that they have no conflict of interest.

Note scientifique sur l'expansion d'une aire de répartition d'un bourdon sédentaire (*Bombus hortorum*) en Nouvelle-Zélande

Bombus hortorum / ***Bombus ruderatus*** / **bourdon / écologie moléculaire**

Eine wissenschaftliche Notiz über die Ausbreitung einer ortsgebundenen Hummel (*Bombus hortorum*) in Neuseeland

Bombus hortorum / ***Bombus ruderatus*** / **Hummel / molekulare Ökologie**

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