

Investigating the molecular phylogeography of New Zealand *Baeocera*

(Scaphidiinae: Coleoptera).

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Phylogeography aims to understand the spatial arrangements of genetic lineages, allowing insight into the evolutionary histories of taxonomic groups. Through the coupling of geographic and genetic information, it is possible to test various evolutionary hypotheses at the intra- and inter-specific level. As a result of continued technological advances, development of new statistical approaches, and reduced costs in obtaining genetic sequencing data, the study of phylogeographic relationships has rapidly increased in the past twenty years, providing further insight into evolutionary processes (Avice, 2009).

The New Zealand biota presents itself as an ideal group to study phylogeographic patterns, due to the detailed knowledge of past geological change and the resulting diverse suite of habitat types available. As such, the origin and events leading to subsequent diversification has been investigated for a variety of New Zealand endemic taxa (Buckley et al., 2001; Baker et al., 2005; Apte et al., 2007; Chapple et al., 2009). For instance the relatively rapid uplift of the Southern Alps in the late Miocene has been found to be an important processes in explaining species diversity of different alpine flora and fauna (Buckley & Simon, 2007).

Understanding the phylogeography of a group is also greatly important from a taxonomic perspective, to understand the systematic relationships between species. The aim of the present research was to investigate the inter- and intra-specific genetic variation of New Zealand native *Baeocera* Erichson, (Staphylinidae: Coleoptera), collected from different geographic regions, using the mitochondrial cytochrome c oxidase (COI) gene. From the data collected, a preliminary understanding of the molecular phylogenetics of this group was obtained, and with further research an understanding of how this group has diversified at a genetic level may be understood.

Study group

The rove-beetle subfamily, Scaphidiinae (Staphylinidae: Coleoptera), is a monophyletic, widespread group occurring on all continents except Antarctica (Löbl & Leschen, 2003). Globally, there are more than 1,400 species of Scaphidiinae, 23 of which have been described from New Zealand, belonging to five genera: *Cyparium* Erichson, *Scaphisoma* Leach, *Baeocera*, *Notonewtonia* Löbl & Leschen and *Brachynopus* Broun, (Löbl & Leschen, 2003).

Baeocera consists of 258 described species worldwide, with 12 species represented in the New Zealand fauna (Löbl & Leschen, 2003). This genus is distinguished by the other four genera through the possession of an aciculate terminal maxillary palpomere and presence of profemoral ctenidium. Although *Baeocera* occur across both the North and South Islands, diversity is centred at the northern region of the South Island, where several species of similar morphology occur. For these species, referred to as the “Nelson group”, identification can only be made through dissection and examination of the genitalia (Löbl & Leschen, 2003). The Nelson group is localised in the northern region of the South Island, which was largely influenced by the uplift of the Southern Alps, and it may be expected that genetic variation of these species may correspond to local biogeographic processes corresponding to distributional ranges and species limits.

Methods

All samples used for genetic analyses were stored in 90-100% ethanol and provided by Richard Leschen. Specimens were first identified using the key from Löbl & Leschen (2003). All specimens were identified to species, except those that could only be identified as the “Nelson group”. The “Nelson group” represents the five species of *Baeocera* that require examination of genitalia for identification. The most morphologically distinct species of *Baeocera* is *B. actuosa* (Broun), which is easily identified by a greatly elongated of antennomere 11. Due to this distinct feature, destructive methods DNA extraction was used for all samples of *B. actuosa*. For all other species, however, non-destructive DNA extraction methods were used, so that specimens could be re-examined post-sequencing if necessary.

Samples of *B. actuosa* were crushed then digested at 56°C overnight hours in 420µL of tissue digest DXT and 4.2µL of DX Digest Enzyme before DNA extraction. DNA was then extracted using the X-tractor Gene™ CAS-1820 (Corbett Life Science). The mitochondrial COI gene region was amplified using the primers CI-J-2195 and TL-N-3014.

Polymerase chain reaction (PCR) amplifications were performed using 25µL reaction volumes. PCR cycling conditions were set at the following conditions: 95°C for 4 minutes, 40 cycles of 94°C for 30 seconds, 45°C for 30 seconds and 72°C for 10 minutes, with samples held thereafter at 10°C. To determine if amplification was successful, the PCR product was run on a 1.5% agarose gel with TAE buffer, then stained with GelRed and observed under UV light.

The extraction methods of samples using non-destructive methods of DNA extraction involved soaking entire specimens of *Baeocera* in 420µL of DXL buffer and 4.2µL of DX Digest Enzyme for at least 24 hours. Thereafter, the same protocol for *B. actuosa* samples was followed for extraction

and amplification, except the reaction volume was 20 μ L, with the amount of DNA included increased (from 2 μ L to 5 μ L). These conditions failed to result in amplified DNA suitable for sequencing for roughly half of all non-destructive extraction samples, and many samples contained only degraded DNA. The small size of specimens combined with the non-destructive extraction methods were thought to produce in these results, so thereafter specimens were slightly crushed prior to digestion.

Amplified PCR product was then purified and sequenced in both directions using a BigDye™ Terminator Cycle Sequencing Ready reaction Mix version 3.1 kit (Applied Biosystems, USA) and then analysed on a 3100 Avant-Genetic Analyser (Applied Biosystems, USA). Sequences were then edited and aligned using Geneious (version 7.0.5, Biomatters) to investigate the phylogeographic patterns for the available samples.

Phylogenetic analyses

In total, 54 sequences representing five identified species and one species complex (Nelson group) were obtained. A phylogenetic tree for all samples based on the COI gene sequences is presented in Figure 1. Although clear groupings between species are not apparent, two broad groupings, one including the Nelson group, *B. punctatissima*, *B. tekootii* and *B. epipleuralis* and another including *B. actuosa* and *B. abrupta* can be seen. The Nelson group is placed in three different groups while *B. tekootii* is located in two separate branches. These data indicate that some of these taxa may be paraphyletic and their species taxonomies require further study. In these instances, COI data needs to be corroboration with genitalic morphology and additional gene sequences. Three groupings for the Nelson group were found between samples collected from (1) North Canterbury, Westland and North Nelson, (2) Marlborough and Kaikoura, and (3) the Marlborough Sounds. The low number of successful DNA amplification for samples using non-destructive methods meant it was not possible to thoroughly investigate the genetic variation of *Baeocera* species that constitute the Nelson group, and this group would be of particular interest as an area for future research.

A preliminary tree showing the genetic variation based on sampling location for all samples of *B. actuosa* is shown in Figure 2. Three major haplotype groups are present in *B. actuosa*, with two in the lower North Island and one widespread. Apart from a Northland group, broad biogeographic groupings are not observable for *B. actuosa*, although some groupings based on location are present. For instance, a distinct separation between all two collected specimens from Kapiti Island (Crosby code – WN; Crosby et al., 1998) and all other samples analysed can be seen. As *B. actuosa* is one of

only two flighted species of *Baeocera* found in New Zealand (the other being *B. abrupta*), the pattern observed may reflect dispersal capability.

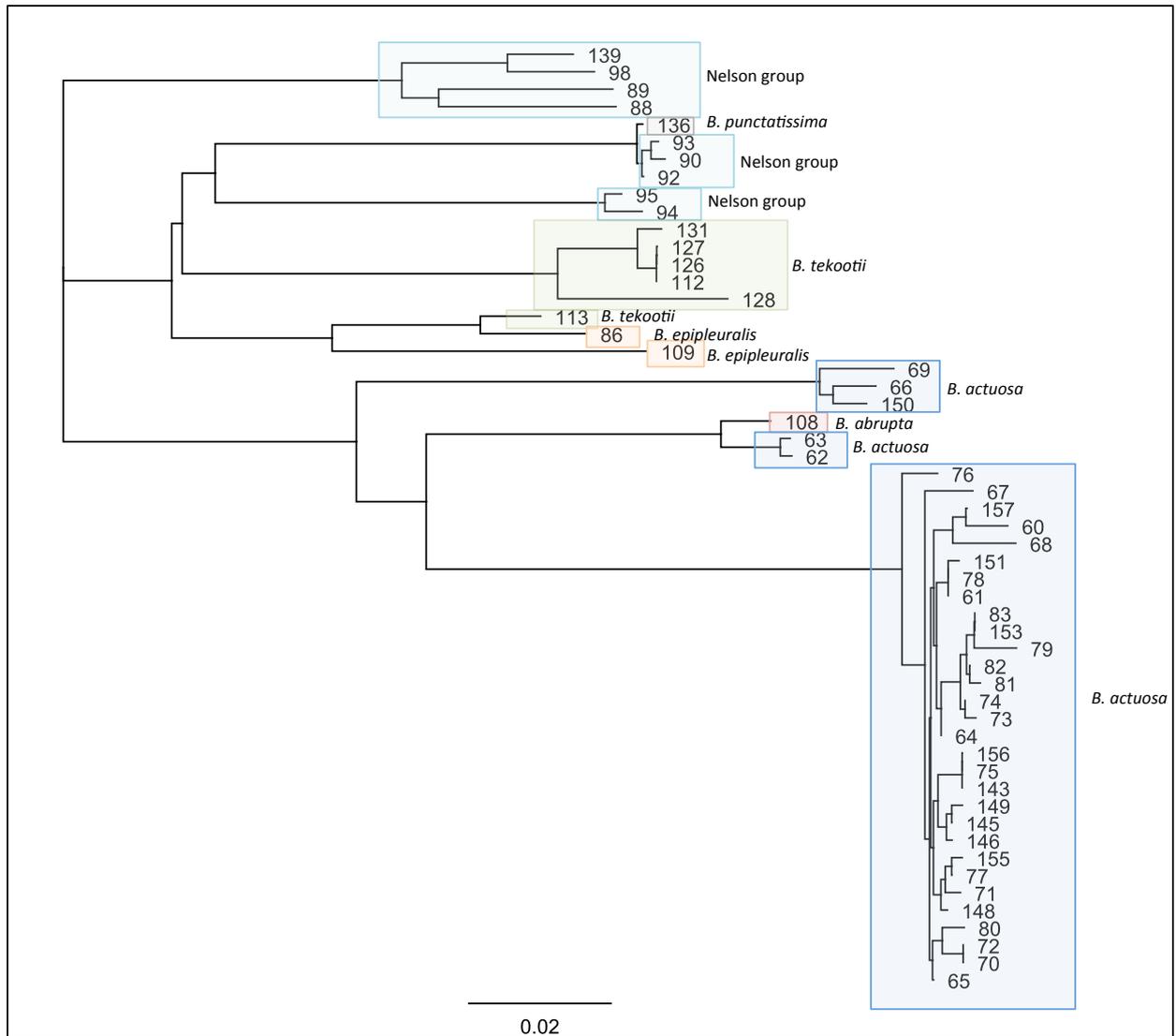


Figure 1. Neighbour-Joining tree for COI gene region for species in the genus *Baeocera*. “Nelson group” refers to the group of five species found in the upper South Island.

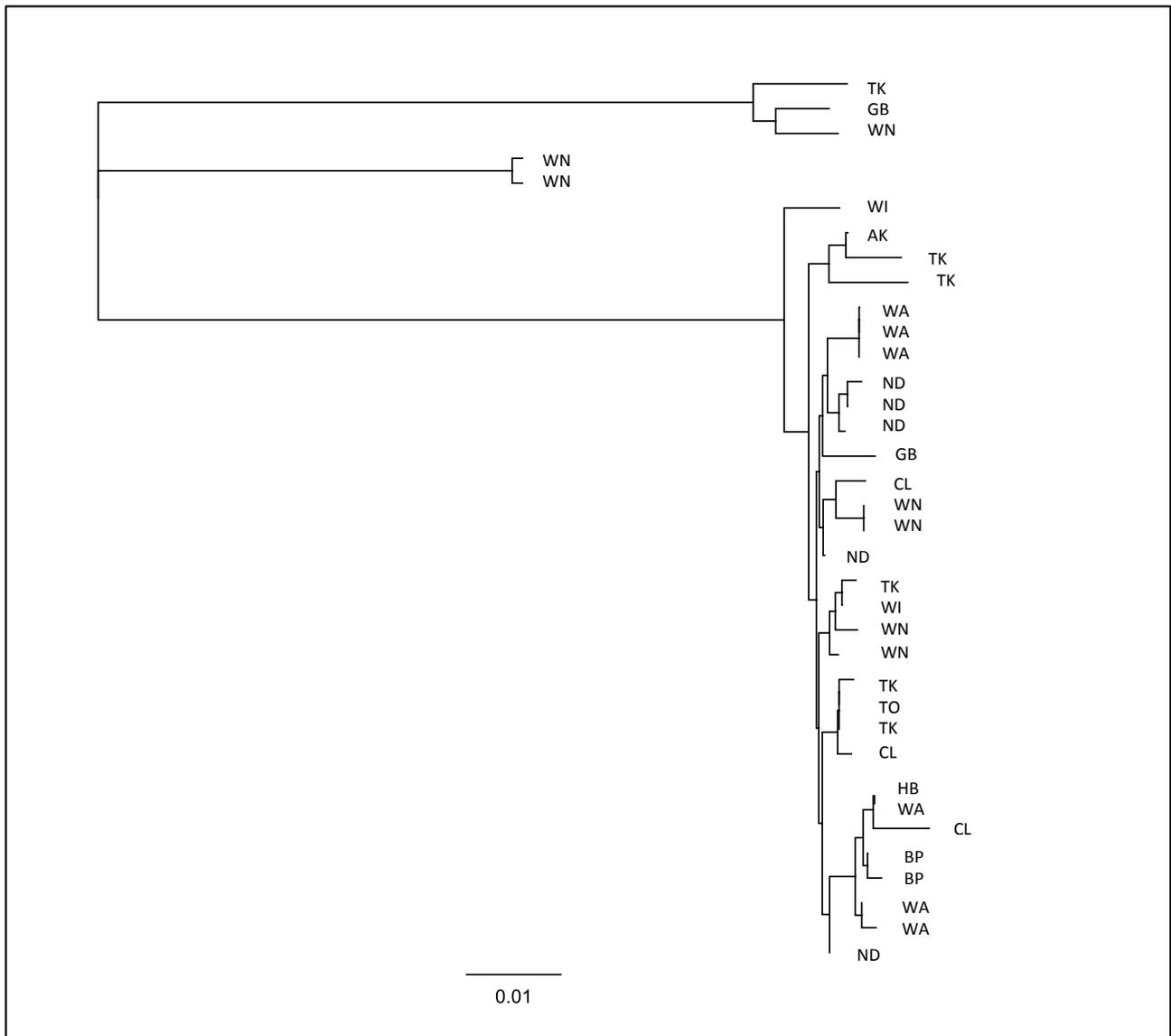


Figure 2. Neighbour-joining tree for COI gene region for species of *B. actuosa*, showing geographic differences based on Crosby codes. TK-Taranaki, GB-Gisborne, WN-Wellington, WI-Wanganui, AK-Auckland, WA-Wairarapa, ND-Northland, CL-Coromandel, TO-Taupo, HB-Hawkes Bay, BP-Bay of Plenty.

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References

- Apte, S., Smith, P.J., Wallis, G.P. (2007). Mitochondrial phylogeography of New Zealand freshwater crayfishes, *Paranephrops* spp. *Molecular Ecology*, 16(9): 1897-1908.
- Avise, J.C. (2009). Phylogeography: retrospect and prospect. *Journal of Biogeography*, 36: 3-15.
- Baker, A.J., Huynen, L.J., Haddrath, O., Millar, C.D., Lambert, D.M. (2005). Reconstructing the tempo and mode of evolution in an extinct clade of birds with ancient DNA: The giant moas of New Zealand. *Proceedings of the National Academy of Sciences*, 102(23): 8257-8262.
- Buckley, T.R., Simon, C. (2007). Evolutionary radiation of the cicada genus *Maoricicada* Dugdale (Hemiptera: Cicadoidea) and the origins of the New Zealand alpine biota. *Biological Journal of the Linnaean Society*, 91: 149-435.
- Buckley, T.R., Simon, C., Chambers, G.K. (2001). Phylogeography of the New Zealand cicada *Maoricicada campbelli* based on mitochondrial DNA sequences: ancient clades associated with Cenozoic environmental change. *Evolution*, 55(7): 1395-1407.
- Chapple, D.G., Ritchie, P.A., Daugherty, C.H. (2009). Origin, diversification, and systematics of the New Zealand skink fauna (Reptilia: Scincidae). *Molecular Phylogenetics and Evolution*, 52: 470-487.
- Crosby, T.K., Dugdale, J.S, Watt, J.C. (1998). Area codes for recording specimen localities in the New subregion. *New Zealand Journal of Zoology*, 25(2):175-183.
- Geneious v.7.0.5 created by Biomatters. Available from <http://geneious.com/>
- Löbl, I & Leschen, R.A.B. (2003). Scaphidiinae (Insecta: Coleoptera: Staphylinidae). *Fauna of New Zealand*, 48, 94pp.