Summer Studentship Report: Molecular Phylogenetics of New Zealand Weevils

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Introduction

Weevils (Curculionidae) make up the most diverse beetle family in New Zealand (Leschen et al., 2003). They are relatively unstudied, and consequently there are gaps in our knowledge of their systematics, biogeography and evolutionary history. The focus of this study is *Geochus*: a genus of weevil common in forest leaf litter throughout New Zealand. There are 22 described *Geochus* species, however the taxonomy for the genus is unresolved and several unclassified morphospecies have been recognised. Species of this genus are observed in a variety of litter types (Kuschel, 1990). Moeed and Meads (1986, 1987) found *Geochus* species in both native beech and broadleaf-podocarp forest litter, though it was noted that they were more abundant in the former. Additionally, Kuschel (1990) observed *Geochus similis* in beach litter. Despite their abundance, little is known about this genus.

Molecular phylogenetic analysis uncovers data that allows inferences to be made about the biogeography and evolutionary relationships of study taxa (Chambers et al., 2001). This project will involve gathering genetic data from seven *Geochus* morphospecies for two gene regions: the relatively conserved nuclear ribosomal RNA gene 28S, and the more variable mitochondrial cytochrome oxidase I (COI). This will be used to produce phylogenetic trees to reveal the biogeographical and species relationships of these morphospecies, and to contribute to the growing available information of the weevils of New Zealand.

Methods

The *Geochus* specimens used in this study were stored in 95-100% ethanol prior to extraction and were sorted into samples by their species and the locations at which they were collected. Numbers were used to classify the morphospecies, and area codes defined by Crosby et al. (1998) to categorise localities. Mixtures made up of 420 µL of tissue digest DXT and 4.2 µL of digest enzyme were used to digest an individual from each sample. The extraction process depended on the number of specimens available. For samples containing more than five individuals destructive extraction methods were used, where whole specimens were crushed in the digest mixture and incubated at 56°C for between 12 and 24 hours. Non-destructive extractions were conducted for samples with five individuals or fewer. This involved soaking entire specimens in the digest mixture at 56°C for 36 – 50 hours, and later removing them to be preserved in ethanol for possible future study. DNA was extracted from the digests using the X-tractor Gene^{TM} CAS-1820 (Corbett Life Science).

The primers LCO1490 and HCO2198 (Folmer et al., 1994) were used to amplify a region of the cytochrome oxidase I (COI) gene. The reaction volumes for PCR were 25 µL, which

included 2.5 µL of DNA for destructively extracted samples or 5 µL for non-destructively extracted ones. PCR conditions for destructively extracted samples were an initial denaturation cycle at 95°C for 4 minutes, then 38 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds and extension at 72°C for 45 seconds, followed by a final extension cycle at 72°C for 5 minutes. Non-destructively extracted samples followed these conditions but with 40 cycles of denaturation, annealing and extension. Gel electrophoresis was then used to determine whether amplification was successful. Samples were run on a 1.5% agarose gel with TAE buffer using a GelRed stain. Bands were then visualised under UV light. Where samples did not amplify PCR was conducted again with 40 cycles, an annealing temperature of 45°C and the volume of DNA added to the reaction mixture was increased to 5 µL where possible.

The region of the 28S gene was amplified using the primers 28S-238-F 5'-CAGAATCGCTACGGACCTCC-3' and 28S-1106-R 5'-AGTCGGGTTGCTTGAGAGTG-3', which were designed using Primer3 version 2.3.4 (Untergasser et al., 2012) as implemented in Geneious version 7.1.5 (http://www.geneious.com, Kearse et al., 2012). The reaction volumes for PCR were the same as for COI, but the PCR conditions were slightly altered: an annealing temperature of 55°C was used, as well as a final extension cycle of 10 minutes. Samples were run on a gel as those were for COI.

Samples that had amplified successfully were sequenced in both directions on the 3130xL Genetic Analyzer (Applied Biosystems) using the BigDye^{TM} Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Consensus sequences were generated by assembling and editing the forward and reverse sequences in Geneious. Geneious was again used to create a multiple alignment for each gene region for phylogenetic analysis. A Neighbour-Joining tree was produced for each gene with the Geneious Tree Builder and the Tamura Nei genetic distance model.

Results and Discussion

Of the 163 samples attempted 140 consensus sequences were obtained and included in the phylogenetic tree for COI, and 110 for 28S. Samples that were identified as being of a new genus similar to *Geochus* were used as the root of the phylogenies (WEV-391 - 394). Most COI samples from species one repeatedly failed to amplify, possibly due to mismatches in the primer binding regions. If this is the case then alternative primers would be needed for additional research in this area.

The COI phylogeny shows that the majority of the *Geochus* sp. 2, 5 and 6 samples group together into their own clades (Fig. 1). In some cases, such as the clade including WEV332, 386, 235 and 385, different species group by their locality. Misidentification or lab error could be responsible for some samples not grouping by their assigned morphospecies number and this needs to be investigated further. There are two distinct clades for *Geochus* sp. 6, indicating that this group could potentially be divided into two species. One clade

contains specimens from localities across the South Island. The other spans the North Island and upper South Island, and though this clade shows more sequence divergence, this raises questions about the dispersal of this species between islands. WEV244 and 351-353 of *Geochus* sp. 2 from the Northland region form a clade that is distinct from the other samples of the same morphospecies and area code. *Geochus* sp. 9 from the Mid Canterbury region appears to be the most divergent, though only one sample was available for this study. The *Geochus* sp. 3 samples were found to be similar to *Geochus* sp. 5 ones in the same regions. The three samples from Three Kings Island (WEV233, 330 and 384) form a clade.

The 28S phylogeny showed much neater groupings of morphospecies (Fig. 2). The *Geochus* sp. 6 samples only form one clade in the 28S tree. As is seen in the COI tree, the 28S samples WEV244 and 351-353 form a separate clade to the other samples of this morphospecies and area. The *Geochus* sp. 9 sample also shows marked sequence divergence for 28S. However the Three Kings Island samples are not all grouped together in the 28S tree, with WEV233 of *Geochus* sp. 1 being a part of a clade otherwise composed of *Geochus* sp. 5 samples from the Southland, Nelson and Wellington regions.

The results of this project have provided a framework for the research into the biogeographical and species relationships of these *Geochus* morphospecies, and will assist in resolving the taxonomy of *Geochus*. The phylogenies will be examined further by comparing the morphology of specimens used to that of vouchers in the New Zealand Arthropod Collection.

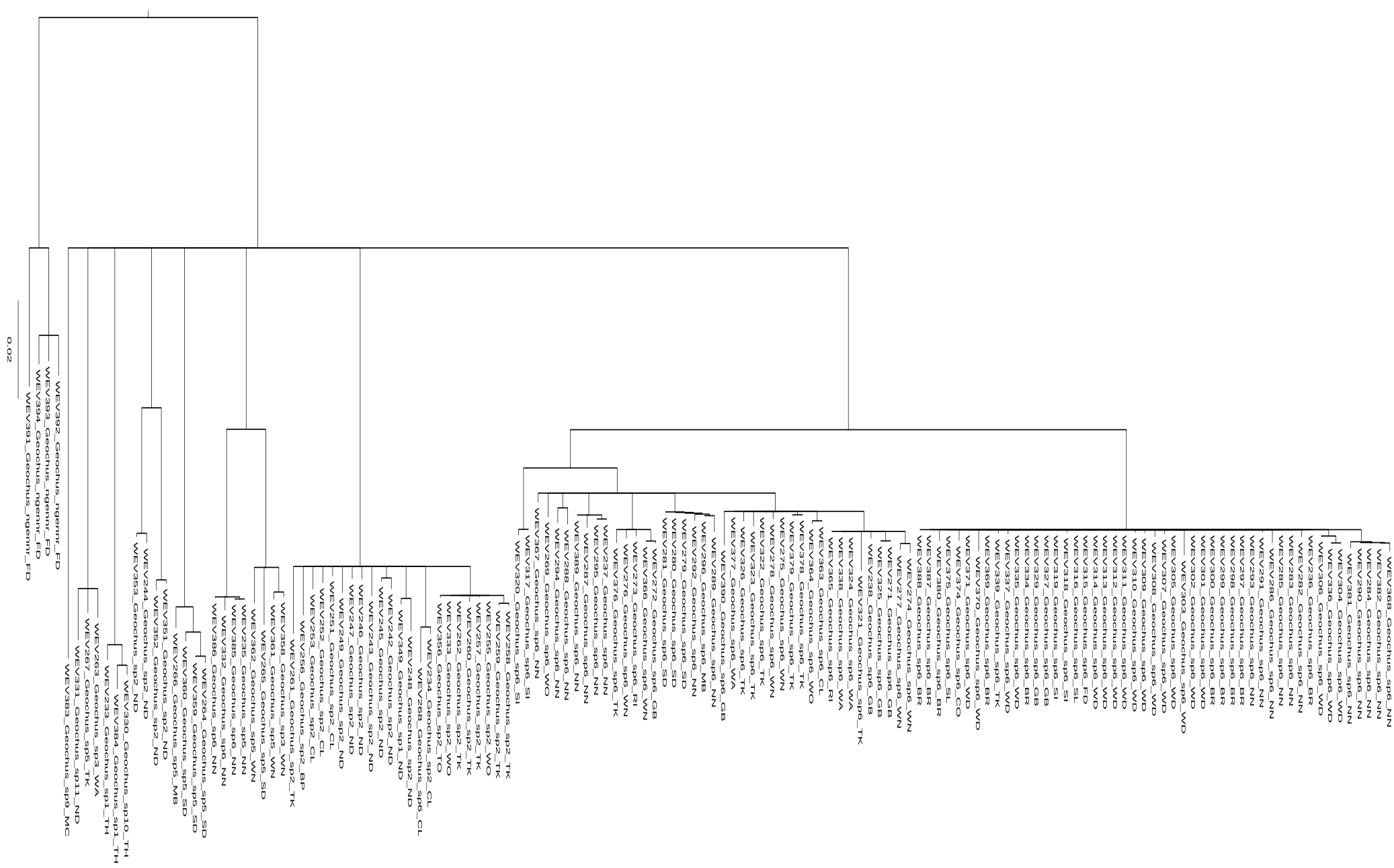

Fig. 1. The Neighbour-Joining phylogeny for the Cytochrome Oxidase I (COI) gene region of 140 Geochus specimens. Locations of sample origin are shown as area codes defined by Crosby et al. (1998).

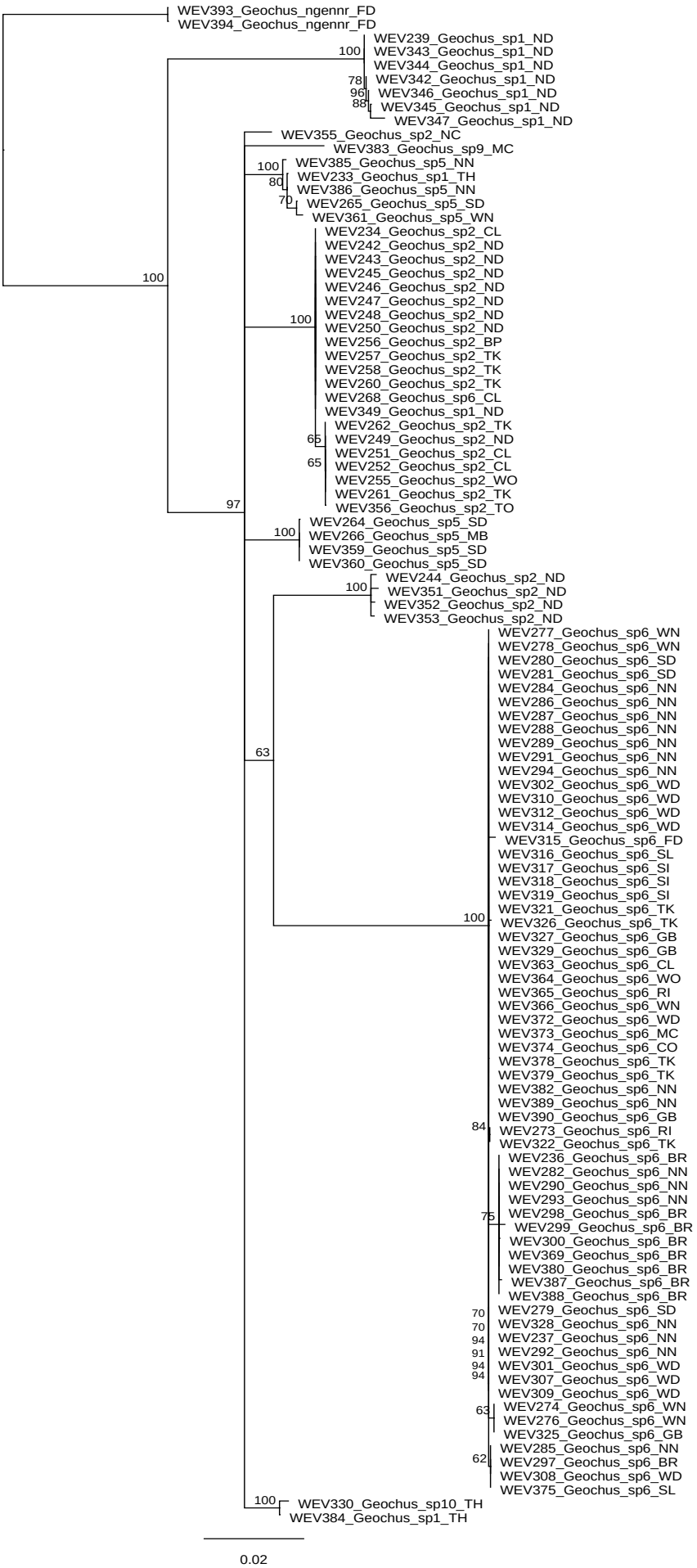

Fig. 2. The Neighbour-Joining phylogeny for the 28S gene region of 110 Geochus specimens. Locations of sample origin are shown as area codes defined by Crosby et al. (1998).

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References

Chambers, G. K., Boon, W. M., Buckley, T. R., & Hitchmough, R. A. (2001). Using molecular methods to understand the Gondwanan affinities of the New Zealand biota: three case studies. *Australian Journal of Botany*, *49*(3), 377-387.

Crosby, T. K., Dugdale, J. S., & Watt, J. C. (1998). Area codes for recording specimen localities in the New Zealand subregion. *New Zealand Journal of Zoology*, *25*(2), 175-183.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*(5), 294-299.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647-1649.

Kuschel, G. (1990). *Beetles in a suburban environment: a New Zealand case study. The identity and status of Coleoptera in the natural and modified habitats of Lynfield, Auckland (1974-1989).* Auckland: New Zealand Department of Scientific and Industrial Research.

Leschen, R. A., Lawrence, J. F., Kuschel, G., Thorpe, S., & Wang, Q. (2003). Coleoptera genera of New Zealand. *New Zealand Entomologist*, *26*(1), 15-28.

Moeed, A., & Meads, M. J. (1986). Seasonality of litter-inhabiting invertebrates in two native-forest communities of Orongorongo Valley, New Zealand. *New Zealand journal of zoology*, *13*(1), 45-63.

Moeed, A., & Meads, M. J. (1987). Seasonality and density of litter and humus invertebrates in broadleaf-podocarp and hard beech forests in Orongorongo Valley, New Zealand. *New Zealand journal of zoology*, *14*(1), 51-63.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—new capabilities and interfaces. *Nucleic acids research*, *40*(15), e115-e115