A Phylogenetic Revision of the New Zealand Endemic Ground Beetle Genus *Oregus* Putzeys 1868 (Carabidae: Broscini)

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S.M. Pawson

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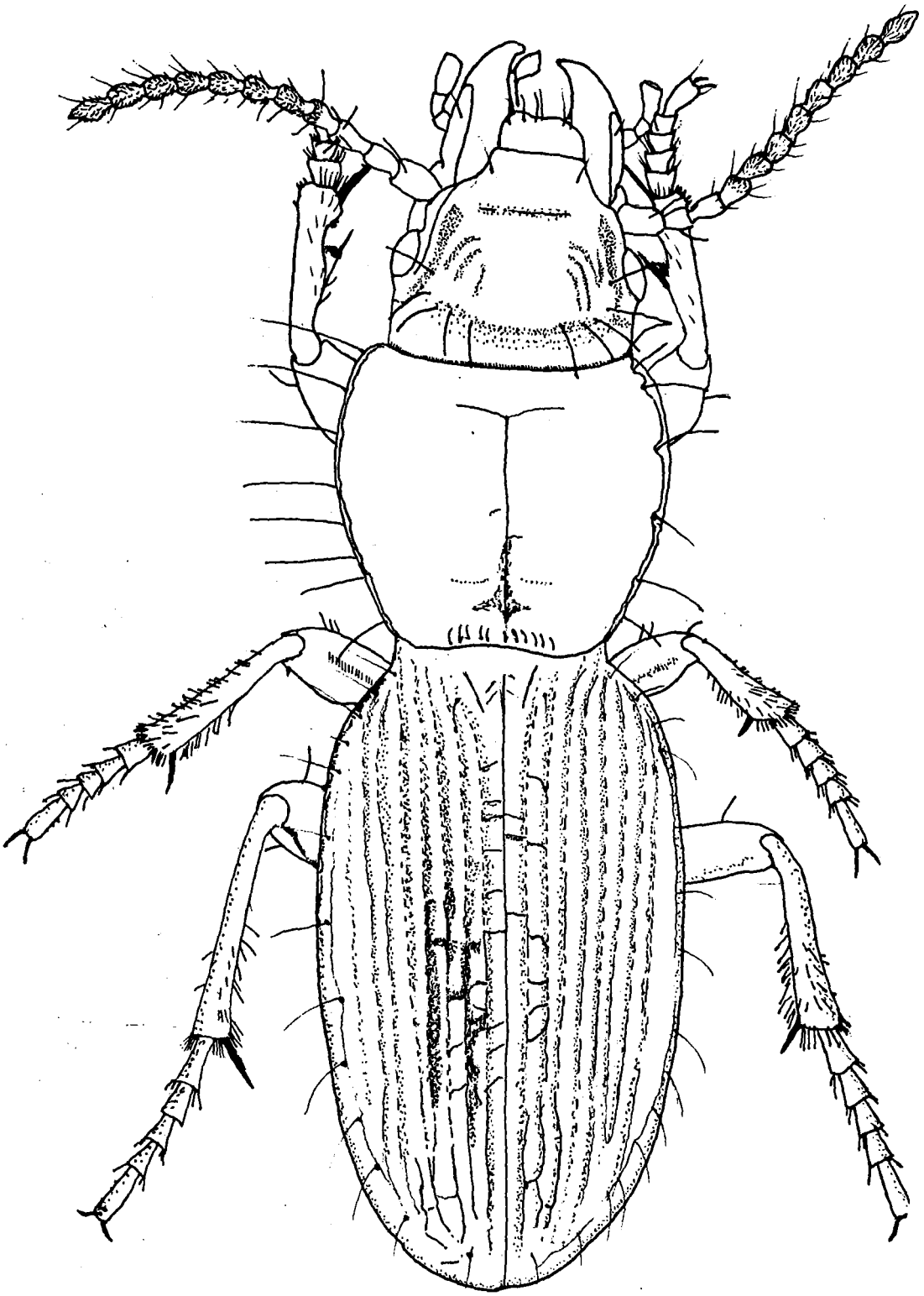

Oregus inaequalis Castelnau 1867, illustrated by S.M. Pawson, 9.5 x life size

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of M.Appl.Sc.

A Phylogenetic Revision of the New Zealand Endemic Ground Beetle Genus *Oregus* Putzeys 1868 (Carabidae: Broscini)

by S.M. Pawson

The genus *Oregus* is an endemic broscine ground beetle restricted in distribution to the east coast of the South Island of New Zealand. The taxonomic and phylogenetic relationships within the genus *Oregus* Putzeys and the abundance and distribution of *Oregus inaequalis* Castelnau were examined. A cladistic analysis of external morphological and genitalic characters was conducted, as well as genetic analysis using partial cytochrome oxidase I and NADH-dehydrogenase I mitochondrial DNA sequences. A total of 2,196 specimens were examined during the course of this study. Specimens were examined from the entire geographic range of the genus. The cladistic analysis was conducted from 17 populations for the morphological characters and 12 populations for the DNA sequences.

Analysis of morphological characters indicated that male genitalic characters were less homoplasious than external morphology. Parsimony analysis of morphological data separated populations of *Oregus* into four species; *O. aereus* White, *O. inaequalis* Castelnau and two new species, subsequently described as *O. septentrionalis* n. sp. and *O. crypticus* n. sp. Mitochondrial DNA sequence data (analysed using parsimony and maximum likelihood) supported the morphological species designations, except for *O. crypticus* as fresh material for DNA analysis of this species was not available. Genetic diversity between species was between 3.05 and 5.36% across both gene regions. Intraspecific genetic diversity was generally low, except in *O. aereus*, which had extensive variation between populations (up to 2.48%). The genetic diversity in *O. aereus* was not reflected in genital morphology.

An extensive pitfall trapping trial failed to collect enough individuals of *O. inaequalis* at Swampy Summit (Dunedin) to allow accurate estimation of abundance using either mark-removal or

mark-recapture methods. However, the number of *O. inaequalis* caught, the low probability of capture and the ratio of *O. inaequalis* caught to other species of Carabidae would still indicate a relatively large population at Swampy Summit. There was an apparent contraction to the geographical range of *O. inaequalis* based on the historical literature, which is merely a reflection of several misidentified specimens. Presence/absence pitfall trapping did not extend the historical distribution of *O. inaequalis* and confirmed that *O. inaequalis* is a narrow range endemic, restricted to the podocarp broadleaf forests and moist tussock/shrubland ecosystems immediately to the north of Dunedin City. Pitfall trapping did not show any significant contraction to the confirmed range of *O. inaequalis*.

Oregus. inaequalis, though a distinct taxonomic entity is not regarded as threatened given the lack of range contraction and the apparently substantial population at Swampy Summit. As such it is not recommended as being a candidate for active conservation management.

Key words: *Oregus*, phylogenetics, parsimony, maximum likelihood, Carabidae, New Zealand, distribution, taxonomy, abundance, mark-removal.

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Chapter 1 General Introduction

History of the genus *Oregus*

The genus *Oregus* Putzeys is a group of endemic New Zealand broscine ground beetles. Since its initial description in 1868, *Oregus* has been expanded to include two nominal species. *Oregus aereus* White is a widely distributed species. Specimens in historical collections were collected from Wellington to Invercargill and along the length of the South Island east of the main divide. *Oregus inaequalis* Castelnau has a highly restricted distribution. The majority of *O. inaequalis* were collected from the Dunedin peri-urban area (the region just beyond the Dunedin metropolitan area) with one specimen from Invercargill (Britton, 1949). There are also references to possible populations of *O. inaequalis* at Fox's Peak, South Canterbury and Lake Pukaki (Ian Townsend personal communication, cited in Jamieson, 1999). *O. inaequalis* is also known to occur in sympatry with *O. aereus* on Swampy Summit, which is part of the Dunedin peri-urban area (Jamieson, 1999). Little is known about the biology or ecology of *Oregus* spp., they are likely to be carnivorous/omnivorous ground foragers, with fossorial tendencies.

Molloy and Davis (1994) ranked *O. inaequalis* as a category B (second priority) species as part of their prioritisation for conservation of New Zealand's threatened fauna and flora. In response to this, the Department of Conservation, Otago Conservancy, commissioned a report entitled, "Existing records of the carabid beetle *Oregus inaequalis* Castlenau in coastal Otago" (Jamieson, 1999). Jamieson's report was a very preliminary investigation based on the collation of existing information about known specimens. This was carried out without visual examination of specimens and included a literature search for references to the genus *Oregus*. Jamieson also dissected the male genitalia of four specimens of *Oregus* (3 *O. aereus* and 1 *O. inaequalis*) collected within Dunedin city. She determined that there was significant variation in the shape of the aedeagus suggesting the possibility of further undescribed species and concluded that the conservation status of these possible new species was critical. A recommendation to the Department of Conservation was subsequently submitted for urgent work, both distributional and taxonomic, to be carried out in the Dunedin urban area.

The distribution of *O. inaequalis* as taken from the literature seems improbable. All recent specimens have been found in the immediate vicinity of Dunedin. It seems unlikely that historical specimens from Invercargill and South Canterbury were *O. inaequalis*. In approximately 150 years of collecting only a handful of specimens from South Canterbury and a single specimen from Invercargill have been collected of what is reputedly *O. inaequalis*. One would have thought such a large conspicuous carabid would have been collected more frequently if it existed in those areas. A thorough examination of historical specimens was critical to verify the historical distribution, as outlying populations would have high conservation value. Swampy Summit has the only currently known population of *O. inaequalis* (Jamieson, 1999). Jamieson (1999) states that this population appears stable, although she provides no justification for this.

Phylogenetic Systematics-The Science

"A common origin implies some similarity" is true, while the converse, "some similarity implies a common origin," is false.... (Dupuis, 1984/pg. 6)

Understanding the phylogeny of a genus is an integral part in determining its taxonomic and biogeographical relationships, hence the phylogenetic approach of this study. Systematics is the science of inferring two processes, cladogenesis and anagenesis. Cladogenesis is the branching of lineages (whether they be individuals, populations or species), anagenesis; is the subsequent genetic divergence that occurs in these split lineages (Futuyma, 1998). Willi Hennig (1966) recognised that the best relationships for classifying groups of organisms were genealogical, i.e., the branching of lineages. He termed this method, phylogenetic systematics, otherwise known as cladistics, *sensu* Mayr (1969). Hennig's method for recovering the evolutionary history of a group of taxa relied on characters he termed apomorphies. Synapomorphies (shared derived characters) are used in phylogenetic systematics to define monophyletic groups of organisms (an ancestral species and *all* of its descendants) (Kitching *et al.*, 1998). Nested sets of monophyletic groupings can be expressed as a cladogram, otherwise known as a phylogenetic tree. This was the crux of Hennig's methodology, the creation of nested sets of taxa grouped by shared derived characters and the use of these phylogenetic trees (cladograms) for the classification of living organisms (Futuyma, 1998). Phylogenetic trees represent a hypothesis of the inferred pathways of

evolution. These hypotheses rely on certain assumptions, which are inherent in all methods, including parsimony and maximum likelihood (Huelsenbeck and Crandall, 1997). These assumptions must be justified (likelihood ratio tests have already been developed to test some of these assumptions (Huelsenbeck and Crandall, 1997)) by criteria beyond mere characters (Kitching *et al.*, 1998). The justification of these assumptions has been a source of heated debate, especially between proponents of various tree-building methodologies.

There are many different classes of characters that can be used to infer relationships between organisms, e.g., morphological, behavioural, ecological, chemical and molecular (protein and DNA). Much attention has focused on the relative merits of molecular sequence and morphological data (Hillis, 1987; Patterson *et al.*, 1993). Historically, morphological characters have dominated phylogenetic systematics, due to the relative simplicity of visual examination. The contribution of molecular sequence data has gained momentum as technology has improved. DNA analysis now plays a major role in phylogenetic systematics where morphological data are inconclusive, deficient, non-existent or poorly analysed.

There is still debate regarding morphological and molecular characters and their accuracy at inferring the path of evolution. Hennig's methodology uses shared derived characters to produce a phylogenetic tree, which is a hypothesis of evolution. Hennig envisaged this hypothesis (and the principal of monophyletic clades) to be used for the classification of all living organisms. Morphological characters are very important in phylogenetic analyses as the majority of species identifications rely on the visual examination of specimens and not molecular based diagnostic techniques. I see molecular characters as an excellent additional data source that can be used to assess the accuracy of phylogenetic trees produced by morphology, i.e., the concept of congruence and morphologically conserved taxa, as is the case of many groups of carabids.

Tree Building Methods

There are many different tree building methods available to infer the phylogenetic history from a given character set. Comprehensive reviews include Swofford *et al.* (1996), Kitching *et al.* (1998), Page and Holmes (1998) and Nei and Kumar (2000). Tree building methodologies are often evaluated by five criteria, efficiency, power, consistency, robustness, and falsifiability

(Penny *et al.* 1992; Hillis 1995). Most tree building methods use a double optimisation approach; initially optimising a single tree based on a specified optimality criterion, such as parsimony or likelihood. The second optimisation involves finding which tree topology from the set of all possible trees is maximum for these criteria (which can involve significant computational problems) (Swofford *et al.*, 1996). This thesis utilises two of the more standard methodologies for tree building, parsimony and maximum likelihood.

Parsimony

Parsimony aims to produce a tree that reconstructs the evolutionary history of all characters, subject to the constraint of invoking the fewest possible changes (Futuyma, 1998; Page and Holmes, 1998; Nei and Kumar, 2000). In doing so, one maximises the amount of similarity that can be explained by homology, whilst minimising the amount of homoplasy (parallel, convergent and reticulate evolution). Parsimony is appealing because it makes few assumptions about the processes of evolution (Page and Holmes, 1998). However, if these assumptions are incorrect, parsimony can still be misleading. Fewer assumptions are preferable as the assumptions are often untestable without reference to the results of the analysis (Mitter, 1981), which is often cited as circular reasoning, though some see this as reciprocal illumination (Hull, 1965; 1988).

Probably the most widely stated objection to the use of parsimony is its reported inconsistency (inability to converge on the correct phylogenetic tree given increasing amounts of data), when different lineages have unequal rates of evolution (Felsenstein, 1978). It is also thought that unequal edge lengths (long tree branches united by short branches, or rapid evolution (Hendy and Penny, 1989; Penny *et al.*, 1992)) are another prime cause of inconsistency. However, Kim (1996) showed that unequal branch lengths were a poor indicator of inconsistency; instead, high rates of evolutionary change and the inclusion of large numbers of taxa in the analysis were much better predictors of inconsistency in the parsimony method. In certain cases, parsimony can be made consistent by the use of appropriate nonlinear transformations to adjust for multiple substitutions of particular characters (Steel *et al.*, 1993).

Maximum Likelihood

Maximum likelihood differs most significantly from other tree building methods by the incorporation of an explicit probabilistic model of evolution (Schoniger and von Haeseler, 1995; Swofford *et al.*, 1996, Page and Holmes, 1998). Rather than mapping characters on phylogenetic trees

(as in parsimony) likelihood methods find the probability that a phylogenetic tree could be produced, given a particular data set and the constraint of a particular model of evolution. The maximum likelihood tree is that which has the greatest probability out of the set of all possible trees. The first attempts to use maximum likelihood for phylogenetic analyses were by Edwards and Cavalli-Sforza (1964), however modern methods of maximum likelihood stem from the seminal work of Felsenstein (1981) and his 'pruning algorithm'. For more recent reviews of maximum likelihood see Swofford *et al.* (1996), Page and Holmes (1998) and Nei and Kumar (2000). Because maximum likelihood methods require an explicit probabilistic model of the evolutionary process they are generally only applied to DNA sequence data for which evolutionary models have been developed. However, new models are being developed to allow the use of maximum likelihood methods on discreet morphological characters (Lewis, 2001).

Maximum likelihood has a good statistical basis (Yang *et al.*, 1994) and allows the testing of many of its assumptions (Goldman, 1993a, 1993b; Huelsenbeck and Crandall, 1997). Different models of sequence evolution have been developed as approximations of evolutionary processes. Initial models, such as, the Jukes and Cantor model (Jukes and Cantor, 1969) assumed that all substitutions occurred at the same rate and that base frequencies were equal. However, it was recognised that such restrictions were overly simplistic (Swofford *et al.*, 1996). More complex models that included unequal base frequencies, multiple substitutions classes and variable rates between nucleotide sites were developed, e.g., HKY85 (Hasegawa *et al.*, 1985) and GTR (General Time-Reversible (Yang, 1994)) (for a detailed discussion see Swofford *et al.* (1996)).

The inclusion of an explicit model of evolution is a point of controversy. On the positive side it requires a precise statement of assumptions (Page and Holmes, 1998). However, there are many models to choose from and some are better estimates of evolutionary history than others, i.e., they may, or may not incorporate factors such as invariable sites, variable base frequencies and variable evolutionary rates, which may, or may not be appropriate for the data set being analysed. The likelihood ratio test (Posada and Crandall, 1998) is the most common method for choosing the most appropriate model and assessing when a model is incorrect for a particular data set (and thus liable to produce inaccurate estimates of phylogeny).

Sources of Conflict

There are a number of sources of conflict that can arise throughout the process of infering evolutionary phylogenies. These can generally be grouped into three sources: a) intra-methodological conflict, i.e., multiple equally parsimonious or equally likely tree topologies, b) inter-methodological conflict, i.e., differences in tree topology between tree construction methods that are greater than expected from sampling error, c) conflict between different data partitions, e.g., different gene regions, or molecular and morphological data partitions.

a) Intra-methodological conflict is very common in phylogenetic analysis. Most authors report a number of equally parsimonious alternative tree topologies (Maddison, 1994). The most common solution to this problem is to look at the consensus of nodes between different tree topologies. Two methods are often used, (i) strict consensus produces a phylogram that is resolved only at nodes consistent across all of the equally parsimonious/likely tree topologies and (ii) the more commonly used majority rule consensus (Margush and McMorris, 1981) which resolves nodes that are consistent across a preset percentage of all equally parsimonious/likely tree topologies. Majority rule consensus trees are normally resolved for consistency set at 50%. It is important to realise that consensus trees do not necessarily represent the most parsimonious/likely representation of character information. Inconsistent branch edges are collapsed when constructing consensus trees. More derived species can be misrepresented on a consensus tree by placing them in a group with their sister taxa/taxon. Consensus trees should not be used as the basis of taxonomic classification (de Queiroz *et al.*, 1995), as what may appear to be a monophyletic clade, could in fact represent a paraphyletic assemblage of taxa. For an in-depth critique of consensus methods, see Kluge and Wolf (1993).

b) Inter-methodological conflict is more difficult to resolve, some differences are to be expected due to the different algorithms and approaches that each method uses. However, major differences such as the rearrangement of clades are not to be expected and need explaining. It is important to realise that congruence between phylogenetic trees, based on different analytical methods such as parsimony and maximum likelihood, indicates the robustness of the phylogenetic signal, as does bootstrapping. Such congruence is often used incorrectly to ascribe a level of accuracy to the phylogenetic tree. To determine the accuracy of a phylogenetic hypothesis it is necessary to investigate congruence between

different data sets (e.g., morphology, different gene regions and behavioural or ecological data), not analytical methods; an important distinction that many fail to realise.

c) The overarching goal of systematics is the discovery of the "true phylogeny" (Brown, 1998; de Queiroz *et al.*, 1995; Miyamoto and Fitch, 1995). However, the outcome of phylogenetic studies are, in reality, merely hypotheses, which represent a best estimate of the true phylogeny (Swofford *et al.*, 1996; Kitching *et al.*, 1998). There are many factors that can bias this estimate of the true phylogeny, such as the reliability of evolutionary models (systematic error) and random or sampling error. The proliferation of different types of data available for phylogenetic analysis has created a further problem; do these different data sets represent equivalent hypotheses of evolutionary history? Also, given the possibility of different modes of evolution, is it appropriate to combine them given our current knowledge of, and available methods for, modelling the pathways of evolution?

Jones *et al.* (1993) and Kluge and Wolf (1993) have argued that data partitions are at best arbitrary and non-existent in nature. Recent molecular studies have illustrated, relatively conclusively, the existence of distinct classes of data that have different modes and thus histories of evolution for the same group of taxa (Bull *et al.*, 1993; de Queiroz *et al.*, 1995; Miyamoto and Fitch, 1995; Sota and Vogler, 2001). There is much debate about whether to analyse data partitions in a combined analysis (total evidence approach), favoured by Kluge and Wolf (1993), or alternatively, to use taxonomic congruence (favoured by Miyamoto and Fitch (1995)). Taxonomic congruence proposes the individual analysis of data partitions followed by an assessment of congruence/consensus between the tree topologies from each data partition. An alternative approach that acknowledges the existence of multiple data partitions and the existence of heterogeneity between such partitions is that of conditional data combination favoured by Bull *et al.* (1993) and de Queiroz (1993; *et al.* 1995). Conditional data combination first tests for heterogeneity between data partitions, if the partitions prove to be homogeneous they are analysed collectively as a single data set, if heterogeneous they are analysed separately. Conceptually it is a different way of looking at the problem; however, analytically it is merely a subset of the total evidence and taxonomic congruence approaches. A number of excellent review articles on these issues have been published including Huelsenbeck *et al.* (1996), Miyamoto and Fitch (1995) and de Queiroz *et al.* (1995).

Most recent researchers tend to take the approach of conditional data combination utilising tests of data homogeneity such as the randomisation test of Rodrigo *et al.* (1993) or, more commonly, the incongruence length difference test (partition homogeneity test, PAUP) (Farris *et al.*, 1995). However, the conditional combination of data errs on the side of total evidence and there is also the issue of type I error rates, i.e., a false positive; rejecting the null hypothesis of data homogeneity when in fact the data partitions are homogeneous. If the incidence of data heterogeneity is <5% ($P < 0.05$) then many cases of heterogeneity identified by such tests will be false positives (Huelsenbeck *et al.*, 1996).

Systematics and the Family Carabidae

Morphology has been the dominant tool used in Jeannel's (1941), Ball's (1979) and Erwin's (1991) seminal papers on carabid classification. External characters have been most commonly used, although some studies have utilised internal characters such as genitalia. Genital characters were thought to be less subject to environmental plasticity (Sharpiro, 1998) and some authors argue that they are a key determinant of reproductive isolation between species. However, insect genitalia are often perceived as species-specific only on the basis that genital differences have been used to determine species groupings (Sharpiro and Porter, 1989). The wide range of new characters examined in recent years has also failed to produce a consensus of the phylogenetic relationships between tribes (Maddison *et al.*, 1999). Because of the inability of morphology to determine the relationships between groups, carabid researchers, e.g., Vogler *et al.* (1993), Vogler and Desalle (1994), Su *et al.* (1996a; 1996b; 1998) and Maddison *et al.* (1999) have begun to utilise molecular techniques such as DNA sequencing to provide characters for phylogenetic analysis.

The use of molecular techniques is now commonplace in modern studies of insect systematics. Such studies have utilised approximately 40 protein-coding genes, all major mitochondrial and nuclear ribosomal RNA genes, and a number of non-coding gene regions (Caterino *et al.*, 2000). However, the majority of genes chosen have been from the mitochondrial genome. Caterino *et al.* (2000) presents a good review of the gene regions used for phylogenetic studies across a broad range of invertebrate taxa. Other reviews that concentrate on the utility of gene regions include,

Simon *et al.* (1994), Kambhampati and Smith (1995), Zhang and Hewitt (1996a, 1997) and for mtDNA and Brower and Desalle (1994) for nuclear gene regions.

Molecular systematics provides a second powerful source of data to examine phylogenetic relationships. A single study using nuclear 18S ribosomal RNA by Maddison *et al.* (1999) attempted to infer the phylogenetic relationships between the tribes of the family Carabidae. Their study, though very detailed in its attempt, could not reliably infer the more basal relationships within the family. Other molecular phylogenetic studies within the family Carabidae have focused on either a single genus or sub-genus, e.g., *Ohomopterus* spp. (Japan) (Sota and Vogler, 2001; Su *et al.*, 1996b), *Damaster* spp. (Japan) (Su *et al.*, 1998), *Cicindela* spp. (Vogler and Desalle, 1994; Vogler *et al.*, 1993), *Patrobus* (Pohl, 1998) and *Carabus* spp. (Pruser and Mossakowski, 1998); or on a closely related group of genera, e.g., the Carabina (Japan) (Su *et al.*, 1996a). The majority of these studies have used mtDNA regions, particularly cytochrome oxidase subunit I (COI), cytochrome oxidase subunit II (COII) and NADH-dehydrogenase subunit 5 (ND5). Studies have utilised nuclear regions and in some cases found a conflict with phylogenetic trees recovered from mtDNA (Sota and Vogler, 2001). Historically nuclear gene regions have been used less frequently than mtDNA. They have a number of different properties to mtDNA, including being biparentally inherited and often single copy. They do however provide a different data source, which may have an alternative evolutionary history that helps identify inaccurate phylogenetic hypotheses produced by the reliance on a single data source like mtDNA.

Because of the demonstrated success of molecular techniques in the field of phylogenetic systematics in general (see Caterino *et al.* (2000)), molecular characters were considered in the present phylogenetic assessment of the genus *Oregus*. There are many molecular techniques and gene regions that can be utilised for phylogenetic systematics; mtDNA gene regions were examined for the reasons outlined below.

Properties of mitochondrial DNA

First used in the late 1970s as a molecular marker (Zhang and Hewitt, 1996b), the analysis of mtDNA has, following the advent of the polymerase chain reaction (PCR), become an incredibly powerful technique in studies of biogeography, phylogenetic systematics, hybridisation, popula-

tion genetics, phylogeography and gene flow (Moritz *et al.*, 1987; Simon *et al.*, 1994; Lunt *et al.*, 1996; Zhang and Hewitt, 1996a, 1996b; Hewitt, 2001).

The applicability of mtDNA for molecular analysis is a reflection of certain properties regarding its structure and mode of evolution (Avise *et al.*, 1987; Moritz *et al.*, 1987; Simon, 1991; Simon *et al.*, 1994; Zhang and Hewitt, 1996b). These include its ease of isolation (pre-PCR work), high copy number within the cell, lack of recombination due to largely maternal inheritance processes (however there is some indication that paternal leakage is more prevalent than previously thought (Kondo *et al.*, 1990; Gyllensten *et al.*, 1991;)), conservation of sequence and sequence structure across a wide range of taxa (allowing development of universal PCR primers, e.g., Simon (1991), Simon *et al.* (1994), Kambhampati and Smith (1995) and Lunt *et al.* (1996)) and the range of evolutionary rates both between and within gene regions (Avise *et al.*, 1987; Moritz *et al.*, 1987; Simon, 1991; Simon *et al.*, 1994; Zhang and Hewitt, 1996a).

However, there are some negative aspects to the use of mtDNA, including heteroplasmy, i.e., multiple forms within a single cell or individual, variable evolutionary rates between genes and taxa, i.e., a non-constant molecular clock (Rand, 1994; Moritz *et al.*, 1987), non-neutrality of substitutions (Moritz *et al.*, 1987) and the recent identification of mitochondrial-like sequences within the nuclear genome (Sunnucks and Hales, 1996; Zhang and Hewitt, 1996b; Bensasson *et al.*, 2001). The possible integration of mitochondrial gene sequences into the nuclear genome is not a recent idea. It was generally accepted over a decade ago that the animal mtDNA has evolved from a larger genome that has been progressively simplified by the transfer of genetic functions to the nuclear genome (Moritz *et al.*, 1987; Gray *et al.*, 1999). However, only recently (in the last five years) have largely inactive mitochondrial-like sequences (pseudogenes) been identified simultaneously in the nuclear and mitochondrial genomes (Zhang and Hewitt, 1996b). Hence the problem of preferential PCR amplification of nuclear pseudogenes rather than target mtDNA sequences, as in Sunnucks and Hale (1996), is of real concern.

Despite some of the negative aspects, the advantages of mitochondrial gene regions make them the most appropriate gene regions for a study of this scale. The disadvantages can be minimised by choosing an appropriate gene region, or detecting pseudogenes as they arise by their particular characteristics, see Bensasson *et al.* (2001).

Thesis Aims

This thesis aims to target the specific research requirements identified by Jamieson (1999), thus allowing the Otago Conservancy of the Department of Conservation to make appropriate decisions for the management of *Oregus*. Research focused on three broad areas:

i) **Determining the species status and species relationships of both *Oregus inaequalis* and *Oregus aereus*.** There has been comment regarding the existence of possible new species of *Oregus* (Jamieson, 1999). However, no in-depth treatment of this group has been attempted since Britton (1949) and the majority of known specimens have been collected since that time. Therefore it is necessary to revise the taxonomy and systematics of this group to allow accurate identification of species. Carabids are morphologically quite conserved and modern techniques such as DNA sequencing are appropriate, on their own or as a test of a morphologically based phylogeny. Assessment of the species status and relationships is described in:

   i. Chapter 2. Morphological review of the genus *Oregus* Putzeys using both external and genitalic characters.

   ii. Chapter 3. Molecular review of the genus *Oregus* using mitochondrial DNA regions COI and ND1.

ii) **Identifying the historical and current distribution of *Oregus inaequalis* and determining the population size of *Oregus inaequalis* at Swampy Summt.** It is important to identify the current distribution of *O. inaequalis* to evaluate the possibility of range contraction, which would indicate the existence of some threat to the survival of the species. Swampy Summit was the only known remaining population of *O. inaequalis* it was critical to have a quantitative estimate of abundance that could be used to monitor future trends in the population. These aspects were considered in Chapter 4.

Chapter 2 Morphological Revision of the Endemic New Zealand Carabid Genus *Oregus* Putzeys 1868 (Coleoptera: Carabidae: Broscini)

Introduction

The genus *Oregus* was first described by Putzeys (1868) for *Broscus aereus* White (type species by original monotypy). White (1846) had previously suggested that *Broscus aereus* might belong to the Australian genus *Promecoderus*. Castlenau (1867), when describing *Mecodema inaequale*, noted its similarity to *Broscus aereus* but retained it in *Mecodema* Blanchard. Putzeys (1873) subsequently moved *M. inaequale* to *Oregus* and Britton (1949) retained this placement of *Oregus aereus* and *Oregus inaequalis* in his comprehensive review of the New Zealand Broscini. Since 1949 there has been no further taxonomic work on this genus. Barbara Barratt (personal communication, 2001) collected a large series of *O. inaequalis* in the mid 1980s, enough to convince other entomologists that *O. inaequalis* was a separate species and not a morphological variant of *O. aereus*. Jamieson (1999), in a brief study of *O. inaequalis*, concluded that there was significant variation in the male genital morphology of *O. aereus* in the Dunedin urban area. This was sufficient, in her opinion, to indicate the possibility of additional species.

Specimens of *Oregus* are most commonly found in south eastern parts of the South Island, and are frequently referred to in surveys of this area, e.g., Patrick (1982), Barratt and Patrick (1987), Dickinson (1988), Patrick *et al.* (1993) and Patrick (1997). Historically, *O. aereus* was recorded from Wellington, WN (as Port Nicholson (White, 1846)) in the North Island and from Motueka, NN, on the north coast of the South Island (Britton, 1949) (two letter abbreviations correspond to the area codes of Crosby *et al.* (1998) for the New Zealand subregion). *O. aereus* is predominantly, but not exclusively, associated with the drier eastern tussock and shrubland communities of South Canterbury and Otago, but is also found in beech forest (*Nothofagus*), mixed beech/podocarp forest and podocarp forests (Larochelle and Lariviere, 2001).

Britton (1949) records *O. inaequalis* from Dunedin, DN, Port Chalmers, DN, Waitati, DN and Invercargill, SL. However, upon detailed examination during this study, the Invercargill specimen turned out to be a specimen of *O. aereus*. Other historical collection records include Swampy Summit, Leith Saddle, Leith Valley, Mount Cargill and Mihiwaka, all localities just to the North of Dunedin City (DN). *O. inaequalis* has been recorded from moist tussock/shrub land communities and kaikawaka (*Libocederus bidwillii*) forest.

This study uses a cladistic approach based on adult morphological characters, both external and genitalic to infer the species relationships within the genus *Oregus*. The analysis also enables the presence of additional undescribed, cryptic, species currently included in *O. aereus* to be determined. A detailed redescription is subsequently given for both *O. inaequalis* and *O. aereus*, and new descriptions are provided for the additional species.

Materials and Methods

Specimen preparation and observation

Morphological characters (both genitalic and external) of adults were examined using a Zeiss stereomicroscope at magnifications of 10, 40 and 62 times. All measurements were taken using an ocular micrometer calibrated with a graticule. Illustrations were drawn with the aid of a camera lucida. Terminalia were removed from specimens after soaking in cold water for 24 hours by the use of fine forceps and dissecting scissors. Excised terminalia were then macerated in cold 10% KOH solution for a further 24 hours and washed with water. The internal sac of the male aedeagus was everted using a fine syringe. Specimens were then further cleansed with water prior to storage in glycerol.

Terminal Taxa Included

Adult characters were examined from populations representing the entire geographic range of the genus. A total of 2,196 specimens were examined and the genitalia of 153 of these were dissected for further analysis. Exemplar specimens were selected from 17 populations for inclusion as terminal taxa in the analysis. Terminal taxa chosen were *O. inaequalis* from Swampy Summit (two individuals chosen to examine the effect of intra-population variation on phylogenetic analysis), DN, *O. aereus s. lat.* from Lake Sedgemere, MB, Hanmer, MB, Culverden, NC, Kelseys Bush, SC, Temuka., SC, Mackenzie Pass, MK, Tasman Valley, Bush Stream., MK, Fox's Peak, MK,

Mt St Bathans, CO, Trotters Gorge, DN, Opoho Bush, Dunedin, DN, DN, Rock and Pillar Range, CO, Remarkables Range, CO, Omeo Huts, Old Man Range, CO and Hokonui Hills, SL. Where possible at least five adult individuals were examined from each population (to observe intra-population variation) and at least two male and two female genitalia dissected. Examination of female genitalia was difficult and some characters were not observed in some populations due to dissection difficulties.

Specimens were examined from institutional and private collections throughout New Zealand and overseas Table 2.1. These are referred to in the text by abbreviations.

Table 2.1. List of private and institutional collections from which specimens of *Oregus* have been examined.

Institutional Collections	**Private Collections and Affiliations**
Auckland Museum (AMNZ)	Barbara Barratt-Agresearch (BIPB)
New Zealand Arthropod Collection (NZAC)	Ian Townsend(ITPC) No Affiliation
Museum of New Zealand, Te Papa (MONZ)	Eric Edwards-Dept of Conservation (EEPC)
Lincoln University (LUNZ)	Peter Johns (PMJ) Canterbury Museum
Canterbury Museum (CMNZ)	
Otago Museum (OMNZ)	
Natural History Museum, London (BMNH)	
Bishop Museum (Hawaii) (BPBM)	
Musei Civico di Storia Naturale, Genova (GMI)	
Museum of Comparative Zoology, Harvard University (MCZ)	
American Museum of Natural History (AMNH)	

Outgroups

The outgroup used in the study was *Diglymma clivinioides. D. clivinoides* was chosen because it is the most geographically widespread member of what is currently regarded as the closest New Zealand genus to *Oregus* (Britton, 1949; Roig-Junent, 2000). The genus *Percosoma* Schaum 1858 (from Australia) was regarded by Roig-Junent (2000) in his cladistic treatment of the world Broscini as the most closely related genus to *Oregus*. Material from the genus *Percosoma* was unavailable for me to examine, hence the use of *Diglymma.*

Characters analysed

A total of 27 characters were used in the final data matrix for cladistic analysis (Appendix A). External adult characters, which have been widely used in other cladistic treatments of carabids, were examined in an attempt to find a suite of phylogenetically informative characters. However a large number of external characters were highly conserved and thus invariable between taxa, this necessitated a reliance on genitalic characters. Attributes of the external features of the aedeagus reported by Britton (1949) were further investigated and these resulted in the inclusion of characters 3 and 18. Ball (1956) first described the presence of internal sclerites X and Y in the subtribe Broscina. Detailed examination of these structures provided a wealth of phylogenetic information, including characters 4-6 and 8-10. Some characters were inapplicable to certain terminal taxa; hence the extra character state of "character not present" was used (Platnick *et al.*, 1991; Maddison and Maddison, 1992).

Method of Cladistic Analysis

Maximum parsimony was used to analyse the data matrix, as it is currently the best technique for examining morphological characters (Maddison and Maddison, 1992). All analyses were conducted in PAUP* (Swofford, 1998) using a heuristic search with the following settings: characters were unordered and *a priori* of equal weight (as no prior information suggested a need for character weighting) (Fitch Optimisation, (Fitch, 1971b)), gaps were treated as "missing", initial trees were obtained by stepwise addition, a tree-bisection-reconnection algorithm was used for branch-swapping and the steepest descent option was not used. Maximum parsimony often produces a number of equally parsimonious tree topologies. Majority rule consensus trees (Margush and McMorris, 1981) were used to summarise such data, combining clades that were common in at least 50 % of all trees. Bootstrapping (1000 replicates) was conducted using equivalent set-

tings for the parsimony search (outlined above) to assess levels of support for individual clades. The nonparametric Kruskal-Wallis test was used to analyse differences between the consistency indexes of external characters and male and female genitalic characters. A similar approach was taken in the analysis of Hepialid moths by Brown (2000).

Results

The geographic range of confirmed collecting localities for the 2196 specimens of *Oregus* examined during this study was from Lake Sedgemere, MB to Invercargill, SL in the South Island. Records of specimens from the North Island and Motueka could not be confirmed, as no specimens with such locality information were located. The material examined is summarised in the species descriptions section (pages 35-43).

The data matrix of 18 terminal taxa and 27 characters is shown in Table 2.2, a description of characters is given in Appendix A. Evaluation of 100,000 random trees produced a G_1= -1.07 that indicates a significant phylogenetic signal (Hillis and Huelsenbeck, 1992). Maximum parsimony analysis produced 90 equally parsimonious trees of 82 steps. These trees were summarised as a majority rule consensus phylogenetic tree (Figure 2.1). A bootstrap analysis (1000 reps) indicated significant support for three branches leading to the terminal taxa *O. inaequalis* and populations of *O. aereus* from Culverden and Hanmer/Lake Sedgemere. A number of synapomorphies characterise the robust branches identified by the bootstrap analysis. However, there was no independent tree to map these characters to, to determine consistency indices (as the molecular tree (chapter 3) did not include all of the populations of *O. aereus* or *O.* "crypticus" and it is inappropriate to map these characters to the morphological tree that identified these synapomorphies (as this would be circular reasoning)).

Table 2.2. Data matrix of 18 terminal taxa and 27 morphological characters. Populations of *O. aereus* are not in italics.

Taxon/Node	111111111122222222 123456789012345678901234567
Diglymma clivinoides	100030000010000010000000200
Oregus inaequalis	602140011000221111110001100
Oregus inaequalis	502240011000221111110001100
Hanmer	311001110111212211121110010
Lake Sedgemere	411021110121212211121110010
Culverden	410111210131312211121021???
Remarkables West Face	401021111111121221111000110
Hokonui Hills	411021111110131221111000???
Rock and Pillar Range	310011111110121221111000202
Kelseys Bush	310121111100121221111000110
Temuka	300121111110121221111000010
Omeo Huts Rd, Old Man Range	411131111201121221111100001?
Trotters Gorge	410021111210131221111000???
MacKenzie Pass	311021111110121221111000010
Fox's Peak	211041111200121231111000010
Mt St Bathans	31103111112?121221111000110
Opoho Bush, Dunedin	411021111110121231111010101
Tasman Valley	311051111100121231111000010

The synapomorphies that delineate the three northern populations (Hanmer, Culverden and Lake Sedgemere) include the presence of setiferous punctures either side of the midline on ventrite 6, (7(0)) (character (character state)) (Figure 2.7B), a single sclerotised projection of the apical plate (14(1)) and the shape of this projection (15(2)), the apical plate is covered in sparse short hairs (20(2)) and the right paramere is setiferous more or less along its entire length (17(1)). The shape of the apical tip of the aedeagus separates these three northern populations into two groups, Hanmer/Lake Sedgemere (23(1)) and Culverden (23(2)). When the entire data matrix (Table 2.2) was analysed the northern species were nested paraphyletically within populations of *O. aereus* (Figure 2.1). The resolution of the consensus phylogram, i.e., no bootstrap support for any populations of *O. aereus*, makes it difficult to determine the actual branching order and the position of the northern clade relative to other populations of *O. aereus*.

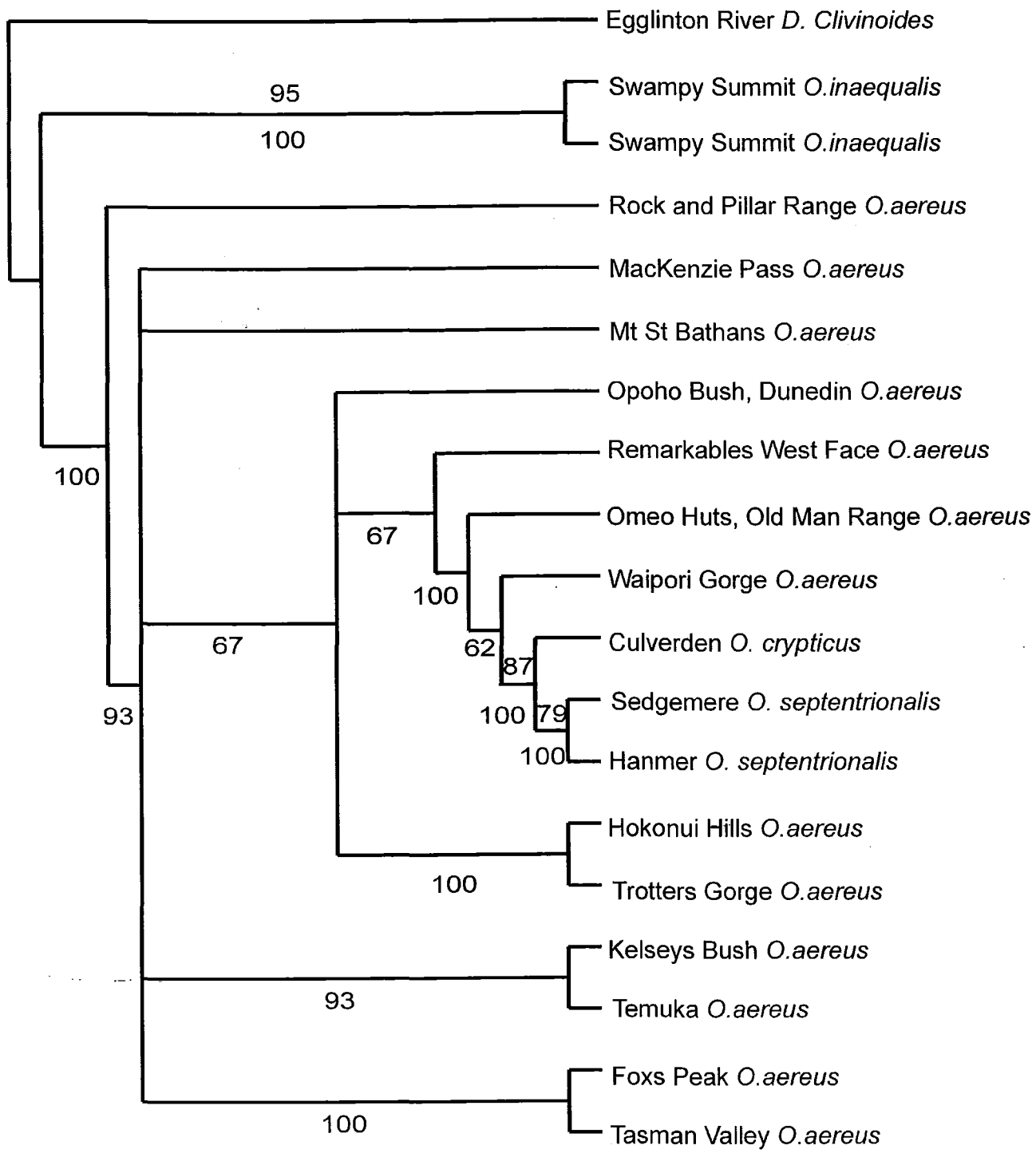


Figure 2.1. Majority rule consensus phylogram of 90 equally parsimonious cladograms. Things Majority rule consensus values are shown below branches; bootstrap support (where greater than 70%) are shown above the branch. Tree length =80 steps, CI= 0.634, RI= 0.634.

However, analysis of a smaller data set, including only male genitalic characters (characters 12-20, 22-24) separated *O. aereus* into a single monophyletic clade, excluding the northern populations (Figure 2.2). Two significant synapomorphies define *O. inaequalis* as a monotypic clade including, moniliform antennae 19(0) (Figure 2.7D) and the lateral extension of the first apical projection 6(1) see Figure 2.6B. *O. aereus* s. str. was much more variable morphologically than populations of *O. inaequalis* or the three northern populations. It also occupied a much greater geographical range (Figure 2.3).

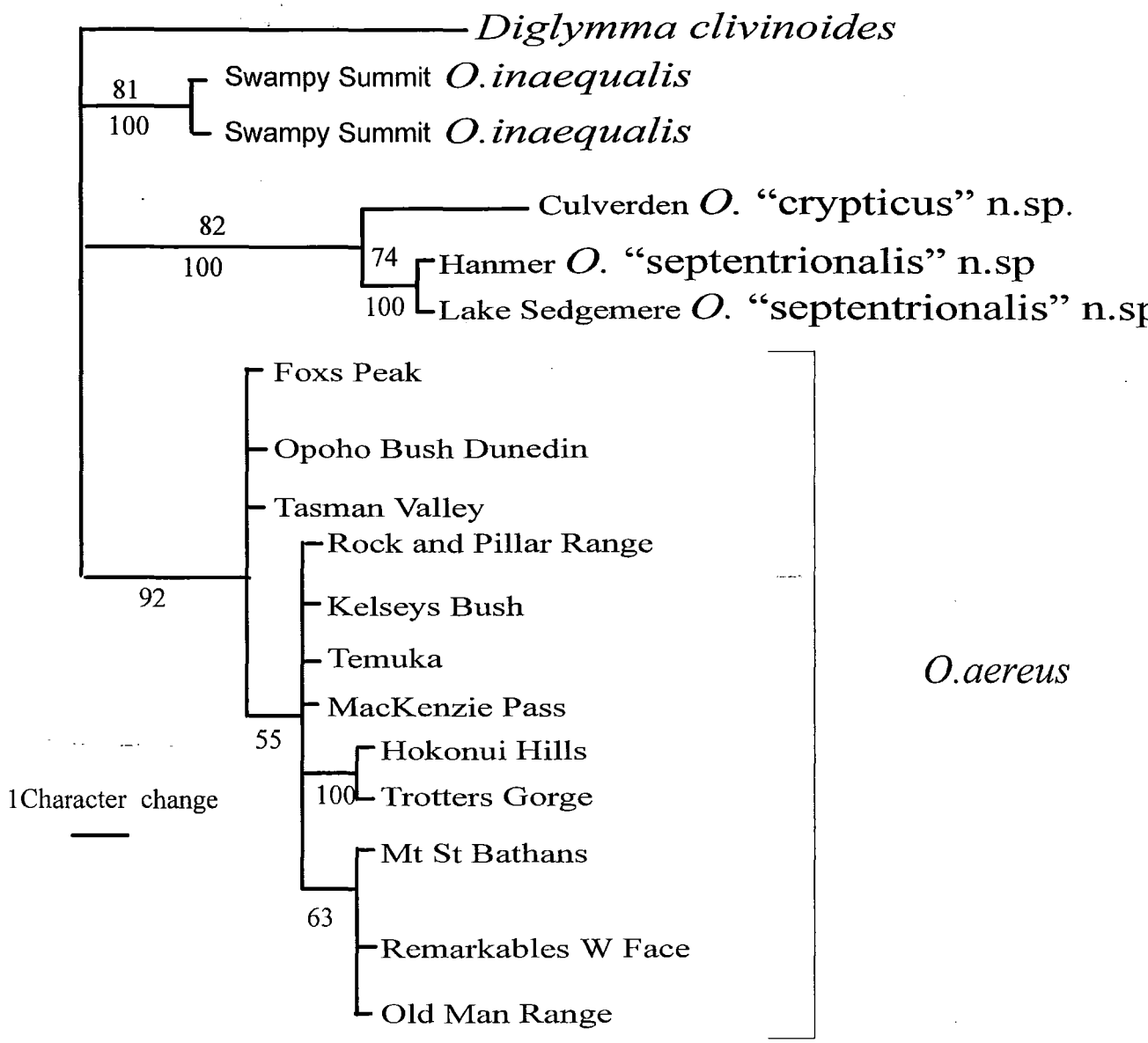


Figure 2.2. Majority rule consensus phylogram of 38 equally parsimonious trees. Tree produced from the analysis of characters in table 2.2, excluding characters 1-11, 21, 25-27. Values below the branches represent majority rule (>50%) consensus values (i.e., a value of 100 means that that

particular branch occurred in 100 % of all 38 equally parsimonious trees); values above branches indicate bootstrap support from 1000 replicates. Tree length = 23 steps, CI= 0.913, RI= 0.923.

The morphological and biogeographic data (Figure 2.3) support the presence of four distinct species, *O. aereus*, *O. inaequalis* and two northern species that are described in the revision of the genus following the discussion below.

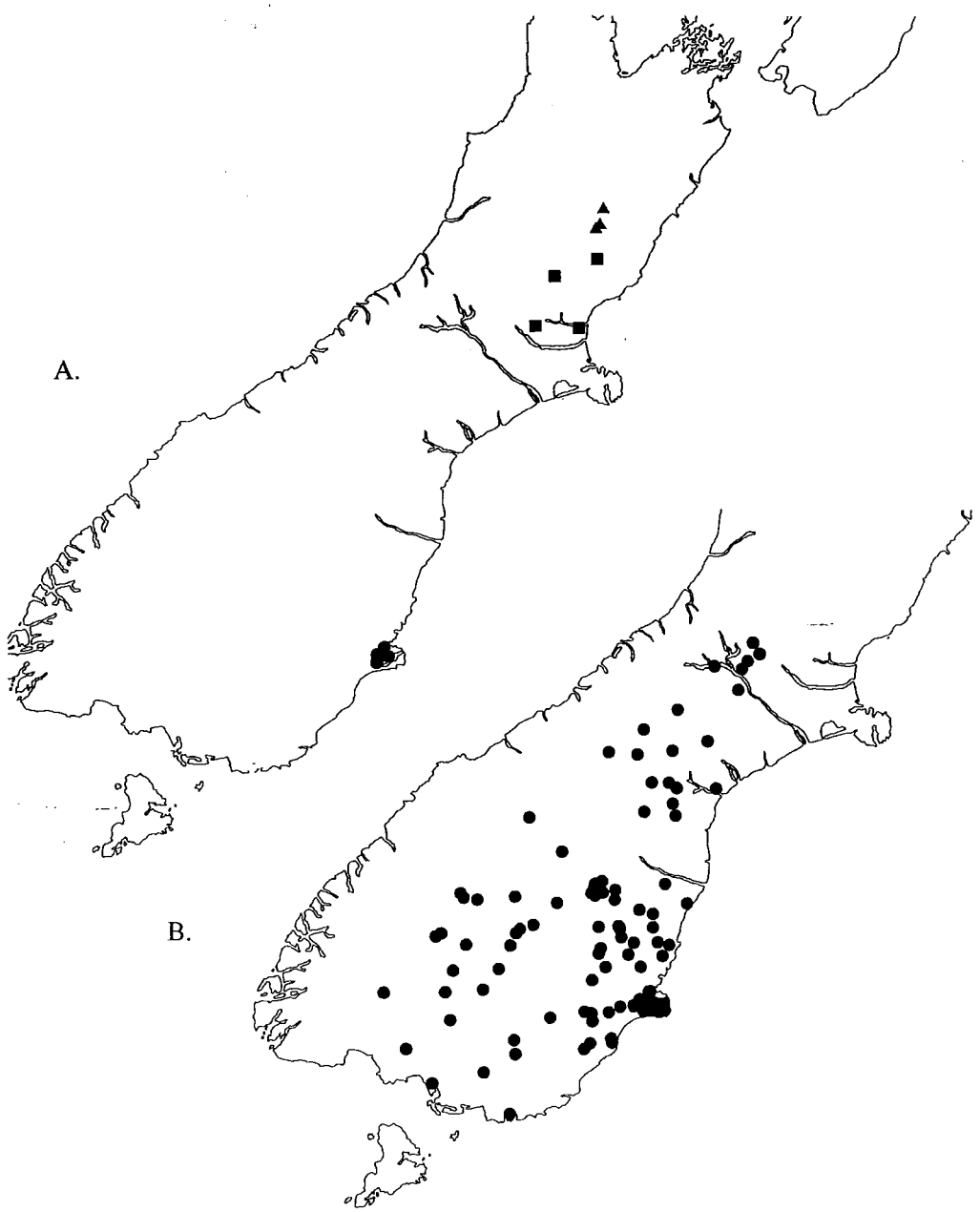


Figure 2.3 A) Distribution of *O. inaequalis* (●), *O. septentrionalis* (▲) and *O. crypticus* (■), B) Distribution of *O. aereus* (●).

Discussion

Based on external characters this group of four carabid species is morphologically highly conserved and the differences between species subtle. These small differences in combination with biogeographic data (Figure 2.3) are, however, sufficient for reliable species identification.

An analysis using all morphological characters (Figure 2.1) indicates that *O. aereus* is intraspecifically more variable (especially the external morphology) than the other three species; this is maybe not surprising considering its much wider distribution (Figure 2.3). However, irrespective of this variability, the eight way polytomy between populations of *O. aereus* produced using male genitalic characters (Figure 2.2) indicates that the morphology of the reproductive organs (of the males at least) is relatively more conserved and suggests a single species.

The separation of *O. inaequalis* as a distinct species based on both morphological and molecular data (chapter 3) supports earlier unpublished research by Barbara Barratt that concluded that *O. inaequalis* was indeed a separate species and not a synonymy. Historical records (Jamieson, 1999; Pawson and Emberson, 2001) in combination with comprehensive searching by Barbara Barratt (pers. comm.) and Pawson and Emberson (2001) indicate that *O. inaequalis* is highly restricted in its distribution. Bearing in mind that "absence of evidence is not evidence of absence" it does seem that this restricted distribution is natural and not an artefact of under collecting or human induced range contraction as it has apparently remained constant in historic times. Thus, the distribution of *O. inaequalis* is interesting not only because of its size, but because the species occurs in sympatry with *O. aereus* (Figure 2.3). However, it seems unlikely that speciation in this instance would have occurred sympatrically. All known localities for *O. inaequalis* are on lava flows of the extinct Miocene Dunedin volcano and it was possibly the action of this volcano that provided the geographic separation necessary for allopatric speciation that was followed by the subsequent re-colonisation of the region by *O. aereus* without hybridisation.

Oregus septentrionalis and *Oregus crypticus* are cryptic species, indistinguishable on the basis of external morphology. The species designations are justified by the markedly different male genitalia and geographic separation. The difference in shape of the tip of the aedeagus (Figure 2.4C and D) is greater than either the intraspecific variability within *O. aereus* (which is minor) or the interspecific variability between *O. aereus* and *O. inaequalis*. The small notch in the ventral sur-

face of the aedeagus of *O. crypticus* indicates a relationship with *O. inaequalis*. The two species appear, based on existing collection records, to be geographically isolated. *O. septentrionalis* is restricted to southern Marlborough and *O. crypticus* to the North Canterbury plains and foothills. The nearest known populations of *O. aereus* are Porters Pass some distance to the south.

The consensus phylogram produced by analysing all characters (Figure 2.1) indicated that *O. aereus* is paraphyletic with respect to the northern species. This is not impossible as these northern species may be an evolutionary offshoot of a particular population, or populations of *O. aereus*. However, their placement (Figure 2.1) as a sister group to populations of *O. aereus* from Central Otago seems unlikely. A close relationship with populations of *O. aereus* from Canterbury or North West Otago would be more plausible. The consensus phylogram produced by the smaller data set (Figure 2.2) is probably a better estimate of the true phylogeny, as the broad divisions are supported by the analysis of mitochondrial DNA (Chapter 3). The paraphyletic arrangement shown in Figure 2.1 is probably a reflection of phylogenetic noise present in some of the external non-genitalic characters.

Of the 27 characters analysed there was considerable variation in their phylogenetic utility. Interestingly, characters derived from male genitalia proved to be the least homoplasious (average consistency index of the 90 equally parsimonious trees, ♂ genitalia=0.860, ♀genitalia=0.467 and external characters=0.656,), especially those associated with the apical plate and sclerites x and y of the internal sac of the aedeagus. The consistency index was significantly different between character partitions as determined by the Kruskal-Wallis test statistic ($K = 632.46$, $P < 0.001$, $df = 2$) when averaged across all 90 equally parsimonious trees. Analysing every tree separately, the majority, 83 out of 90 trees, had significant differences between character partitions. Although inconclusive on its own (the Kruskal-Wallis test statistic) the congruence with molecular data (Chapter 3) indicate that male genetalic characters are more accurate estimators of phylogeny for this group. Other studies of closely related species have found similar results, where male genitalic characters proved the most reliable for infering phylogenies (Brown, 2000).

In the past structures associated with the male genitalia (internal sclerites and plates) were often ignored due to the difficulty of their extraction. Ball (1956) first described them and used their presence as a defining character of the subtribe Broscina. Roig-Junent (2000) also made use of these structures (four characters) in his cladistic analysis of broscine genera. The results reported

here indicate that the finer structure of these sclerites can be used with considerable success in species diagnoses for this group.

Genus *Oregus* Putzeys, 1868

TYPE SPECIES: *Broscus* (*Promecoderus*?) *aereus* White, 1846, by original monotypy.

DESCRIPTION: Head with 2 supraorbital setae; vertex with shallow transverse depression bearing 1-5 setae on each side. Antennae 11 segmented; single setiferous punctures present on segments 1 and 2; segment 3 with apical ring of 8 setae (Figure 2.7C). Apical two-thirds of segment 4 pilose; segments 5-11 fully pilose. Each mandibles with a single tooth. Frontoclypeal suture distinct. Mentum with posterior pair of setiferous punctures; tooth bifid. Submentum with a pair of setiferous punctures on each side. Labial palpi bearing 3 setae on the second segment; palpi widest at apex, apices truncate. Pronotal lateral margin raised, bearing 6-11 setiferous punctures; disc smooth, apart from distinctly impressed midline with anterior and posterior depressions; posterior lateral depressions faint or non-existent. Proepisterna, faintly wrinkled posterior ventral region. Mesepisterna, distinctly wrinkled. Posterior meeting of proepimeron and prosternal process without fusion closes procoxal cavities. Elytral margin raised, bearing 11-19 setiferous punctures; striae faintly to moderately punctate and impressed; parascutellar striole separated from the apical portion of stria 1; parascutellar seta (at the base of the second stria) present. Median lobe of male genitalia sclerotised dorsally in basal one-third, sclerotisation completely enclosing basal orifice, retaining a small weakly sclerotised basal keel. Sclerite X elongate, narrow and abruptly expanded at apex. Apical plate bearing 1-3 sclerotised projections, covered with short hairs. Left paramere with setae in some species; right paramere setiferous from half to full length. Female spermatheca long, thin, joining bursa copulatrix at a broad, flat helmenthoid sclerite. Accessory gland long, thin and apically expanded, positioned on opposite side of the helmenthoid sclerite from junction of the median oviduct and bursa copulatrix. Basal portion of female gonocoxite with 3-4 spines on inner margin.

COMMENTS: Previously included by Ball (1956) in the subtribe Broscina, *Oregus* has been recently reassigned, along with the other New Zealand and Australian genera, included by Ball in

the Broscina, as part of a newly erected subtribe, the Nothobroscina (Roig-Junent, 2000). *Oregus* is easily separated from the other New Zealand Nothobroscina by the arrangement of the supraorbital setae. *Oregus* has a pair of supraorbital punctures on each side, each bearing a single setae while *Diglymma* has a single supraorbital puncture that bears one seta. *Mecodema*, *Metaglymma* and *Brullea* have a single supraorbital puncture with multiple setae.

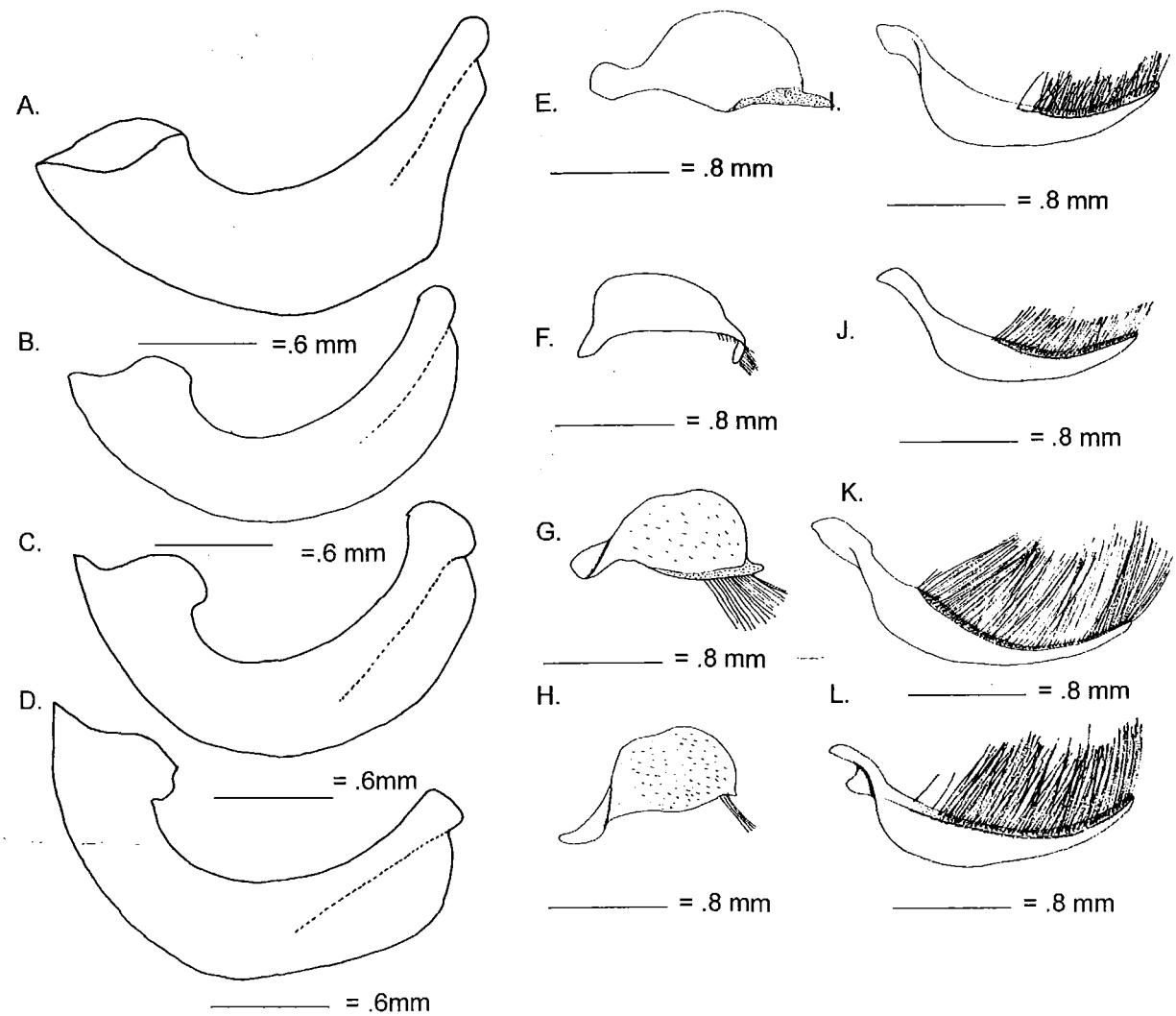


Figure 2.4. A. Adeagus of *O. aereus*, B. Aedeagus of *O. inaequalis*, C. Aedeagus of *O. crypticus*, D. Aedeagus of *O. septentrionalis*, E. Left paramere of *O. aereus*, F. Left paramere of *O. inaequalis*, G.Left paramere of *O.crypticus*, H. Left paramere of *O. septentrionalis*, I. Right paramere of *O. aereus*, J. Right paramere of *O. inaequalis*, K. Right paramere of *O. crypticus*, L. Right paramere of *O. septentrionalis*.

Key to Species

1a. Antennae nearly moniliform (Figure 2.7D); elytral striae deeply impressed and irregular in the apical one-third.....*O. inaequalis* (Castelnau).

1b. Antennae filiform (Figure 2.7C); elytral striae lightly or moderately impressed and regular in form almost to the apex of the elytra.....2

2a Ventrite six without a pair of setiferous punctures either side of the midline .....*O. aereus* (White)

2b Ventrite six with a pair of setiferous punctures either side of the midline (Figure 2.7B) 3

3a Femora and tibia vivid red-brown; tip of the aedeagus distinctly enlarged; rounded (Figure 2.4C).....*O. crypticus* Pawson n.sp.

3b Femora and tibia dull red-brown; tip of the aedeagus distinctly truncate (Figure 2.4D) .....*O. septentrionalis* Pawson n. sp.

Species Descriptions

Note: ♂ and ♀ indicate sex of specimen, where this is not known a symbol is not provided, (W) indicates specimens held in alcohol, a ? in the date indicates that portion of the date was not known.

Oregus aereus (White, 1846)

Original combination: *Broscus (Promecoderus?) aereus* White, 1846:5. Stated type locality: Port Nicholson, WN.

Alternative Combinations: *Promecoderus aereus*: (Chenu, 1851)

Mecodema oeneum {sic}: (Castelnau, 1868)

Mecodema aereum: (Gemminger and Harold, 1868)

Oregus aereus (Putzeys, 1868)

Oregus aeneus {sic}: (Putzeys, 1873)

Holotype: Port Nicholson, WN, Collected Voyage of HMS. Erebus and Terror (type locality disputed, see below) deposited in BMNH.

Other Material Examined: 8km NW Springfield, Waimakariri River, MC, 1, 15.i.1960, BMH., Broken river, MC, 1, ?, MONZ., Porter Ck, Craigeburn Range, MC, 1, 23.iv.1969, NZAC., Mesopotamia, MC, 1♂,

14.v.1957, CMNZ., Double Hill, Rakaia, MC, 1, ?.v.1956, PMJ., Mt Hutt, MC, 1, 1.ii.1962, NZAC., Cameron Valley, Top Hut, MC, 4♂,1♀, 28.xi.1964, CMNZ., Mt Peel, SC, 1♂, 26.i.1964, NZAC., Fox's Peak, SC, 3♀,1♂, 22.x.1963, CMNZ., Fox's Peak, SC, 1, 21.i.1964, NZAC., Mt Nimrod, Sc. Res, SC, 1, 25.xi.1990, LUNZ., Tekapo, Mt John, MK, 1♀, 7.xii.1962, PMJ., Tasman Valley, Bush Stream, MK, 1♀,1, 17.xi.1991, LUNZ., Tasman Valley, Bush Stream, MK, 1♀,1, 31.i.1988, LUNZ., Bush Stream, Mt Cook, MK, 1♀, 2.i.1964, NZAC., Albury, SC, 1♂,4, 3.ii.1932, NZAC., Mt W. of Albury, SC, 1, 23.x.1927, BMNH., Temuka, SC, 2♂,2♀,1, ?.iii.1874, BMNH., Temuka, SC, 1, ?.iii.1874, BMNH., Temuka, SC, 3♀, 6.iii.1966, CMNZ., Temuka, SC, 1, 24.x.1976, CMNZ., Temuka, SC, 1♀, 18.ix.1966, CMNZ., Temuka, SC, 1♀,1, 12.i.1875, CMNZ., Temuka, SC, 1♂, 1.viii.1972, PMJ., Upper Pareora Gorge, SC, 1, 21.iii.1970, LUNZ., Kelseys Bush, SC, 8♂,3♀,1, 23.ix.1962, CMNZ., Kelseys Bush, SC, 1♂, 1.xii.1968, CMNZ., Kelseys Bush, SC, 1, 27.xii.1987, LUNZ., Kelseys Bush, SC, 1♂,1♀, 7.iv.1984, PMJ., Kings Cave, SC, 1, 10.xi.1972, LUNZ., Kings Cave, SC, 1, 1.viii.1971, PMJ., Blue Cliffs, Hunter Hills, SC, 1, 18.iii.1980, PMJ., Limestone Valley, SC, 1, 22.iv.1973, LUNZ., Mt Harper, SC, 1, 14.ii.1962, NZAC., Mt St Bathans, MK, 1♀, 21.x.1964, CMNZ., Kirkliston Range, MK, 1, 22.i.1966, NZAC., Hawkdun Range, Otago, CO, 1, 11.xii.1991-30.i.1992, OMNZ., Haka Saddle, CO, 1, 17.i.1966, NZAC., Steep Grade, CO, 1, 27.i.1968, OMNZ(W)., Mackenzie Monument, MK, 1♀,1♂, 25.x.1976, CMNZ., Mackenzie Monument, MK, 3♀ , 29.x.1964, CMNZ., Mackenzie Pass, MK, 3♀,1♂, 8.v.1966, CMNZ., Mackenzie Pass, MK, 1♂,1♀,2, 10.v.1972, PMJ., Oamaru, DN, 1♀, ?.x.1961, MONZ., Oamaru, DN, 2, ?.x.1963, MONZ., Oamaru, DN, 1, ?.ii.1962, CMNZ., Kyeburn, CO, 1, 5.iii.1989, OMNZ., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 16.viii.1967, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 2, 27.i.1969, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 14.x.1968, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 15.i.1969, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 16.xii.1967, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 14.iii.1968, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 2, 26.xii.1968, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 25.xi.1967, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 2, 12.ix.1968, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 24.iii.1968, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 2, 15.xi.1969, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 27.xii.1967, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 8.v.1968, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 6.xii.1967, OMNZ(W)., Corner Littler Kyeburn, Naseby, Dansey Pass Rd, CO, 1, 15.i.1969, OMNZ(W)., Dansey Pass Rd , CO, 1♂, 15.x.1969, CMNZ., Dansey Pass Rd summit, 3067ft, Central Otago, CO, 2, 3♀, 16.xii.1979, OMNZ., Spec Ck, Kyeburn, CO, 2, 24.ix.1967, OMNZ(W)., Wedderburn, CO, 2, 23.v.1968, OMNZ(W)., Wedderburn, CO, 5, 17.iv.1969, OMNZ(W)., Wedderburn, CO, 3, 15.x.1967, OMNZ(W)., Wedderburn, CO, 3, 14.x.1968, OMNZ(W)., Wedderburn, CO, 1, 27.i.1969, OMNZ(W)., Wedderburn, CO, 6, 15.i.1969, OMNZ(W)., Wedderburn, CO, 14, 20.xi.1968, OMNZ(W)., Wedderburn, CO, 1, 25.xi.1967, OMNZ(W)., Wedderburn, CO, 4, 6.xii.1967, OMNZ(W)., Wedderburn, CO, 2, 14.x.1967, OMNZ(W)., Wedderburn, CO, 3, 6.i.1968, OMNZ(W)., Wedderburn, CO, 6, 16.ii.1969, OMNZ(W)., Wedderburn, CO, 1, 19.iii.1968, OMNZ(W)., Wedderburn, CO, 4, 29.iii.1969, OMNZ(W)., Wedderburn, CO, 1, 15.xi.1967, OMNZ(W)., Wedderburn, CO, 1, 6.iii.1969, OMNZ(W)., Wedderburn, CO, 2, 16.xii.1967, OMNZ(W)., Wedderburn, CO, 3, 24.ix.1967, OMNZ(W)., Wedderburn, CO, 2, 27.i.1968, OMNZ(W)., Wedderburn, CO, 3, 15.i.1969, OMNZ(W)., Wedderburn, CO, 7, 12.xii.1968, OMNZ(W)., Wedderburn, CO, 2, 4.x.1967, OMNZ(W)., Wedderburn, CO, 1, 15.v.1969, OMNZ(W)., Wedderburn, CO, 2, 8.iv.1968, OMNZ(W)., Wedderburn, CO, 3, 24.iii.1968, OMNZ(W)., Wedderburn, CO, 1, ?, BMNH., Mt Ida, CO, 1,1♀, 21.x.1923, MONZ., Naseby, CO, 1, 1♀, 14.xi.1961, OMNZ., Near Idaburn, 600m, CO, 1, 17.iv.1994, OMNZ., Near Naseby forest, CO, 3, 20.xi.1969, OMNZ(W)., Near Naseby forest, CO, 1, 27.ii.1967, OMNZ(W)., Near Naseby forest, CO, 1, 29.viii.1968, OMNZ(W)., Near Naseby forest, CO, 4, 27.i.1969, OMNZ(W)., Near Naseby forest, CO, 3, 14.x.1968, OMNZ(W)., Near Naseby forest, CO, 1, 18.iv.1968, OMNZ(W)., Near Naseby forest, CO, 2, 6.xii.1967, OMNZ(W)., Near Naseby forest, CO, 6, 24.ix.1967, OMNZ(W)., Near Naseby forest, CO, 2, 6.iii.1969, OMNZ(W)., Near Naseby forest, CO, 1, 26.ii.1968, OMNZ(W)., Near Naseby forest, CO, 1, 15.i.1969, OMNZ(W)., Near Naseby forest, CO, 2, 25.x.1967, OMNZ(W)., Near Naseby forest, CO, 3, 12.xii.1968, OMNZ(W)., Near Naseby forest, CO, 3, 15.xi.1967, OMNZ(W)., Near Naseby forest, CO, 4, 25.xi.1967, OMNZ(W)., East Br. Ewe Burn, CO, 5, 23.v.1968, OMNZ(W)., East Br. Ewe Burn, CO, 1, 27.i.1968, OMNZ(W)., East Br. Ewe Burn, CO, 8, 27.i.1969, OMNZ(W)., East Br. Ewe Burn, CO, 4, 26.ix.1968, OMNZ(W)., East Br. Ewe Burn, CO, 16, 20.xi.1968, OMNZ(W)., East Br. Ewe Burn, CO, 5, 6.iii.1969, OMNZ(W)., East Br. Ewe Burn, CO, 5, 14.x.1968, OMNZ(W)., East Br. Ewe Burn, CO, 3, 6.xii.1967, OMNZ(W)., East Br. Ewe Burn, CO, 1, 14.iii.1968, OMNZ(W)., East Br. Ewe Burn, CO, 1, 29.viii.1968, OMNZ(W)., East Br. Ewe Burn, CO, 3, 17.iv.1969, OMNZ(W)., East Br. Ewe Burn, CO, 6, 15.i.1969, OMNZ(W)., East Br. Ewe Burn, CO, 2, 12.ix.1968, OMNZ(W)., East Br. Ewe Burn, CO, 1, 15.v.1967, OMNZ(W)., East Br. Ewe

Burn, CO, 1, 18.iv.1968, OMNZ(W)., Swinburn Bridge, CO, 4, 24.iii.1968, OMNZ(W)., Swinburn Bridge, CO, 2, 27.xii.1967, OMNZ(W)., Swinburn Bridge, CO, 4, 16.ii.1967, OMNZ(W)., Swinburn Bridge, CO, 2, 6.xii.1967, OMNZ(W)., Swinburn Bridge, CO, 1, 1.iii.1968, OMNZ(W)., Swinburn Bridge, CO, 2, 14.iii.1968, OMNZ(W)., Swinburn Bridge, CO, 4, 6.i.1968, OMNZ(W)., Swinburn Bridge, CO, 3, 24.ix.1967, OMNZ(W)., Swinburn Bridge, CO, 1, 20.xi.1968, OMNZ(W)., Swinburn Bridge, CO, 1, 4.xi.1967, OMNZ(W)., Swinburn Bridge, CO, 2, 26.ix.1968, OMNZ(W)., Swinburn Bridge, CO, 2, 25.x.1967, OMNZ(W)., Swinburn Bridge, CO, 2, 15.xi.1967, OMNZ(W)., Swinburn Bridge, CO, 1, 11.vi.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 8, 20.xi.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 5, 17.iv.1969, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 3, 15.v.1969, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 3, 9.xii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 1, 27.xii.1967, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 3, 29.viii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 2, 21.ii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 1, 18.iv.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 5, 14.x.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 3, 26.xii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 2, 1.iii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 3, 6.iii.1969, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 3, 16.ii.1967, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 4, 14.iii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 1, 4.x.1967, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 1, 25.x.1967, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 2, 6.i.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 2, 24.iii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 4, 26.xii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 3, 24.iii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 3, 26.xii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 1, 26.xii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 4, 15.i.1969, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 3, 14.x.1967, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 1, 9.ii.1968, OMNZ(W)., Highway 85, Near Mt Swinburn, CO, 1, 11.vi.1968, OMNZ(W)., Kokonga (1), CO, 13, 24.iii.1968, OMNZ(W)., Kokonga (1), CO, 3, 30.vii.1968, OMNZ(W)., Kokonga (1), CO, 9, 23.v.1968, OMNZ(W)., Kokonga (1), CO, 5, 27.xii.1967, OMNZ(W)., Kokonga (1), CO, 4, 6.i.1968, OMNZ(W)., Kokonga (1), CO, 2, 21.ii.1968, OMNZ(W)., Kokonga (1), CO, 2, 15.viii.1968, OMNZ(W)., Kokonga (1), CO, 7, 14.iii.1968, OMNZ(W)., Kokonga (1), CO, 5, 25.xi.1967, OMNZ(W)., Kokonga (1), CO, 3, 25.xi.1967, OMNZ(W) Kokonga (1), CO, 9, 29.viii.1968, OMNZ(W)., Kokonga (1), CO, 8, 20.xi.1968, OMNZ(W)., Kokonga (1), CO, 7, 12.ix.1968, OMNZ(W)., Kokonga (1), CO, 4, 16.xii.1967, OMNZ(W)., Kokonga (1), CO, 1, 1.iii.1968, OMNZ(W) Kokonga (1), CO, 10, 4.xi.1967, OMNZ(W)., Kokonga (1), CO, 6, 18.iv.1968, OMNZ(W)., Kokonga (1), CO, 10, 6.xii.1967, OMNZ(W)., Kokonga (1), CO, 12, 26.ix.1968, OMNZ(W)., Kokonga (1), CO, 1, 4.xi.1967, OMNZ(W) Kokonga (1), CO, 3, 8.v.1968, OMNZ(W)., Kokonga (1), CO, 15, 14.x.1968, OMNZ(W)., Kokonga (1), CO, 2, 15.i.1969, OMNZ(W)., Kokonga (1), CO, 3, 12.xii.1968, OMNZ(W)., Kokonga (1), CO, 2, 6.iii.1969, OMNZ(W)., Kokonga (1), CO, 1, 9.ii.1968, OMNZ(W)., Kokonga (1), CO, 4, 16.ii.1969, OMNZ(W)., Kokonga (1), CO, 6, 15.xi.1967, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 2, 4.xi.1967, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 2, 15.viii.1968, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 1, 6.xii.1967, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 3, 15.viii.1968, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 1, 23.v.1968, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 2, 6.ii.1969, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 6, 12.ix.1968, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 2, 26.xii.1968, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 2, 26.ix.1968, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 1, 24.iii.1968, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 3, 15.i.1969, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 5, 12.xii.1968, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 1, 6.i.1968, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 2, 26.xii.1968, OMNZ(W)., Summit steep grade., Nth of Tiroiti, CO, 9, 28.xi.1968, OMNZ(W)., Cromwell, CO, 1, 23.x.?, OMNZ(W)., Alexandra, CO, 1♂, 22.x.1964, PMJ., Old Man range, CO, 1♀, 30.xi.1973, LUNZ., Old Man Range, CO, 3♀, 1♂, 21.x.1964, PMJ., Omeo Huts, Old man Range, CO, 2♀,2, 12.ix.1968, NZAC., Tiroiti, Otago, CO, 1, 15.ii.1989, OMNZ., Patearoa, CO, 1, 14.x.1968, OMNZ(W)., Patearoa, CO, 1, 20.xi.1968, OMNZ(W)., Patearoa, CO, 1, 25.xi.1967, OMNZ(W)., Patearoa, CO, 1, 16.ii.1969, OMNZ(W)., Hyde, CO, 1, 16.xii.1967, OMNZ(W)., Hyde, CO, 1, 9.xii.1968, OMNZ(W)., Hyde, CO, 1, 6.i.1968, OMNZ(W)., Obelisk Range, Central Otago, 4100ft, CO, 3, 23.iii.1972, OMNZ., Obelisk Range, Central Otago, 4100ft, CO, 1, 4-11.i.1991, OMNZ., Tawhiti Creek, CO, 1, 29.x.1958, OMNZ(W)., Deep Dell, CO, 2, 14.x.1968, OMNZ(W)., Deep Dell, CO, 5, 4.xi.1967, OMNZ(W)., Deep Dell, CO, 2, 27.i.1968, OMNZ(W)., Deep Dell, CO, 1, 25.xi.1967, OMNZ(W)., Deep Dell, CO, 2, 27.xii.1967, OMNZ(W)., Deep Dell, CO, 2, 6.i.1968, OMNZ(W)., Deep Dell, CO, 1, 18.ix.1968, OMNZ(W)., Deep Dell, CO, 1, 6.iii.1969, OMNZ(W)., ., Deep Dell, CO, 3, 1.iii.1968, OMNZ(W)., Deep Dell, CO, 2, 26.xii.1968, OMNZ(W)., Deep Dell, CO, 2, 15.i.1969, OMNZ(W)., Deep Dell, CO, 14, 24.ix.1967, OMNZ(W)., Deep Dell, CO, 2, 16.xii.1967, OMNZ(W)., Deep Dell, CO, 2, 14.iii.1968, OMNZ(W)., Deep Dell, CO, 3, 4.x.1967, OMNZ(W)., Deep Dell, CO, 1, 14.x.1967, OMNZ(W)., Deep Dell, CO, 2, 21.ii.1968,

OMNZ(W)., Deep Dell, CO, 1, 6.xii.1967, OMNZ(W)., Deep Dell, CO, 4, 25.x.1967, OMNZ(W)., Deep Dell, CO, 4, 24.ix.1967, OMNZ(W)., Deep Dell, CO, 3, 9.xii.1968, OMNZ(W)., Deep Dell, CO, 2, 24.iii.1968, OMNZ(W)., Deep Dell, CO, 7, 20.xi.1968, OMNZ(W)., Deep Dell, CO, 4, 27.i.1969, OMNZ(W)., Horse Range, 240m, DN, 1, 19.ix.1995, OMNZ., Rocklands Tussock, CO, 1, 19-xii.1978, NZAC., Dunback-Macraes Road, CO, 4, 24.ix.1967, OMNZ(W)., Dunback-Macraes Road, CO, 1, 16.viii.1967, OMNZ(W)., Dunback-Macraes Road, CO, 1, 6.iii.1969, OMNZ(W)., Dunback-Macraes Road, CO, 12, 24.ix.1967, OMNZ(W)., Dunback-Macraes Road, CO, 1, 20.xi.1968, OMNZ(W)., Hampden, CO, 1, ?, NZAC., Macraes Flat, CO, 1, 20.iii.1969, OMNZ(W)., Macraes Flat, CO, 3, 6.i.1968, OMNZ(W)., Macraes Flat, CO, 2, 10.iii.1969, OMNZ(W)., Macraes Flat, CO, 1, 24.ix.1967, OMNZ(W)., Macraes Flat, CO, 1, 25.xi.1967, OMNZ(W)., Macraes Flat, CO, 4, 26.xii.1968, OMNZ(W)., Macraes Flat, CO, 4, 21.ii.1968, OMNZ(W)., Macraes Flat, CO, 1, 29.viii.1968, OMNZ(W)., Macraes Flat, CO, 3, 12.xii.1968, OMNZ(W)., Macraes Flat, CO, 1, 4.xi.1967, OMNZ(W)., Macraes Flat, CO, 3, 27.i.1969, OMNZ(W)., Macraes Flat, CO, 2, 24.iii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 19, 24.ix.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 11, 15.v.1969, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 17, 6.i.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 8, 27.xii.1969, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 19, 17.iv.1969, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 20, 27.xii.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 1, 9.vii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 3, 15.viii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 6, 16.i.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 8, 21.ii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 3, 15.viii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 2, 24.ix.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 2, 15.viii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 17, 6.xii.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 5, 26.ix.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 10, 12.xii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 16, 14.xii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 11, 9.ii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 20, 24.ix.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 14, 14.x.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 16, 24.ix.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 18, 24.ix.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 18, 24.ix.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 8, 14.iii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 19, 14.x.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 22, 20.xi.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 12, 20.xi.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 20, 27.i.1969, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 16, 29.viii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 47, 12.ix.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 3, 6.iii.1969, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 4, 23.v.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 2, 27.i.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 8, 16.ii.1969, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 15, 15.xi.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 34, 15.i.1969, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 20, 20.xi.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 21, 18.iv.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 3, 15.viii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 3, 11.vi.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 2, 18.iv.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 19, 8.i.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 74, 26.xii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 9, 24.iii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 23, 4.xi.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 31, 26.ix.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 4, 12.xii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 8, 15.v.1969, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 19, 12.xii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 5, 1.iii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 2, 4.v.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 18, 4.v.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 7, 4.xi.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 13, 14.x.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 19, 25.x.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 16, 16.xii.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 20, 25.xi.1967, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 6, 30.vii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 18, 12.xii.1968, OMNZ(W)., Summit Taieri Ridge, Deep Dell-Fillyburn, DN, 22, 15.i.1969, OMNZ(W)., Mt Totters, Palmerston, DN, 1♀, 12.?.1969, PMJ., Trotters Gorge, DN, 1, , PMJ., Trotters Plain, 350m, DN, 1, 14.v.1994, OMNZ., 1 mile south summit rock, Rock and Pillars, CO, 1, 18.i.1969, OMNZ(W)., 1 mile south summit rock, Rock and Pillars, CO, 3, 18.xii.1968, OMNZ(W)., 1 mile south summit rock, Rock and Pillars, CO, 4, 15.xi.1968,

OMNZ(W)., 3500 feet, Cushion veg Spur , Rock and Pillars, CO, 5, 23.xi.1968, OMNZ(W)., 3500 feet, Cushion veg Spur , Rock and Pillars, CO, 8, 23.xi.1968, OMNZ(W)., 3500 feet, Cushion veg Spur , Rock and Pillars, CO, 1, 20.x.1968, OMNZ(W)., 3500 feet, Cushion veg Spur , Rock and Pillars, CO, 7, 31.xii.1968, OMNZ(W)., 3500 feet, Cushion veg Spur , Rock and Pillars, CO, 5, 20.x.1968, OMNZ(W)., 800 feet, matagouri scrub, Rock and Pillars, CO, 6, 23.xi.1968, OMNZ(W)., 800 feet, matagouri scrub, Rock and Pillars, CO, , 20.x.1968, OMNZ(W)., 800 feet, matagouri scrub, Rock and Pillars, CO, 1, 31.xii.1968, OMNZ(W)., East of summit rock, Rock and Pillars, CO, 1, 18.i.1969, OMNZ(W)., East of summit rock, Rock and Pillars, CO, 3, 18.xii.1969, OMNZ(W)., East of summit rock, Rock and Pillars, CO, 1, 30.iii.1969, OMNZ(W)., East of summit rock, Rock and Pillars, CO, 4, 29.iv.1969, OMNZ(W)., Lug Ck, Rock and Pillars, CO, 2, 29.iv.1969, OMNZ(W)., Lug Ck, Rock and Pillars, CO, 2, 18.i.1969, OMNZ(W)., Lug Ck, Rock and Pillars, CO, 2, 10.iii.1969, OMNZ(W)., Rock & Pillar, South of Summit Rock, 4500ft, CO, 1, 30.x.1971, OMNZ., Rock and Pillar Range, CO, 21, 1968-1969, NZAC., Rock and Pillars, CO, 1♀, 20.x.1964, PMJ., Rock and Pillars, CO, 1♀, 1♂, 20.x.1964, PMJ., Rock and Pillars, CO, 1♂, 14.viii.1965, PMJ., y 1/2 mile N Summit rock, Rock and Pillars, CO, 2, 31.xii.1968, OMNZ(W)., Nenthorn, 500m, DN, 1, 10.iv.1994, OMNZ., Fillyburn Bridge, CO, 1, 24.ix.1967, OMNZ(W)., Fillyburn Bridge, CO, 1, 25.x.1967, OMNZ(W)., West of Middlemarch, 3000 feet Shady Gully, CO, 8, 23.xi.1968, OMNZ(W)., West of Middlemarch, 3000 feet Shady Gully, CO, 2, 8.ii.1969, OMNZ(W)., West of Middlemarch, 3000 feet Shady Gully, CO, 1, 8.ii.1969, OMNZ(W)., W of Middlemarch 2000 ft, CO, 1, 23.xi.1968, OMNZ(W)., W of Middlemarch3500ft, CO, 5, 31.xii.1968, OMNZ(W)., W of Middlemarch3500ft, CO, 4, 23.xi.1968, OMNZ(W)., W of Middlemarch3500ft, CO, 11, 31.xii.1968, OMNZ(W)., W of Middlemarch3500ft, CO, 1, 8.ii.1969, OMNZ(W)., W of Middlemarch3500ft, CO, 5, 23.xi.1968, OMNZ(W)., W of Middlemarch3500ft, CO, 4, 31.xii.1968, OMNZ(W)., Mt Dasher, , 5♂,2♀,1, 17.v.1980, PMJ., Mt Misery Rd, Waianakarua, , 3, 22.v.1971, OMNZ(W)., Sutton, CO, 1♀, 9.ix.1968, NZAC., Waitati, Otago, DN, 5, 5.vi.1990, OMNZ., Doctors Point, Waitati , DN, 1, 23.iii.1913, LUNZ., Woodhaugh Gardens, Dunedin, DN, 1, 7.iix. 1975, OMNZ., Woodhaugh, Dunedin, DN, 3, 1.iv.1994, OMNZ., Woodside, Taieri, DN, 1, 28.x.1923, AMNZ., Mt Cargill, 680m, DN, 1, 28.ix.1984, OMNZ., Broadleaf forest, Leith Saddle, Dunedin, DN, 1, 2-12.ii.1976, OMNZ., Opoho Bush, Cemetery Road, Dunedin , DN, 1, 25-31.i.1971, OMNZ(W)., Opoho Bush, Cemetery Road, Dunedin , DN, 8, 1-15.iii.1971, OMNZ(W)., Opoho Bush, Cemetery Road, Dunedin , DN, 1, 21-28.xii.1970, OMNZ(W)., Opoho Bush, Cemetery Road, Dunedin , DN, 5, 10-17.xi.1970, OMNZ(W)., Opoho Bush, Cemetery Road, Dunedin , DN, 2, 18-25.i.1971, OMNZ(W)., Opoho Bush, Cemetery Road, Dunedin , DN, 3, 14-21.xii.1970, OMNZ(W)., Opoho Bush, Cemetery Road, Dunedin , DN, 1, 4-11.i.1971, OMNZ(W)., Opoho Bush, Cemetery Road, Dunedin , DN, 2, 28.xii.1970-4.i.1971, OMNZ(W)., Opoho Bush, Cemetery Road, Dunedin , DN, 2, 23-30.xi.1970, OMNZ(W)., Opoho Bush, Cemetery Road, Dunedin , DN, 1, 23-30.xi.1970, OMNZ(W)., Flagstaff Creek, 180m, DN, 1, 26.x.1998, OMNZ., Dunedin, DN, 1♂,1♀, ?.?.1877, BMNH., Dunedin, DN, 1, 27.v.1923, BMNH., Dunedin, DN, 1, 23.xii.1927, OMNZ., Dunedin, DN, 1, vi.1908, OMNZ., Dunedin, DN, 2, ?, MONZ., Dunedin, DN, 1, ?.i.1909, MONZ., Dunedin, DN, 1, ?.iv.1960, OMNZ(W)., Dunedin or Catlins Rd, DN, 1♀, ?.x.1940, AMNZ., Dunedin, Portobello, DN, 1♂, 29.viii.1957, PMJ232., Kaikoroi Valley, DN, 1, 30.iv.1995, OMNZ., Bradford, Dunedin, DN, 1, 1.xii.1997, OMNZ., Broad Bay, Otago Penninsula, DN, 1, 3.iv.1994, OMNZ., Cape Saunders, DN, 1, 4.xi.1923, AMNZ., 14 Hilary St, Dunedin, DN, 1, 6.xii.1975, OMNZ., Akatore R., Otago, DN, 1, 10.x.1992, OMNZ., Leith Valley, DN, 2, 4.iv.1994, OMNZ., Okia Flat, Otago Penninsula, DN, 1, 26.iv.1994, OMNZ., Swampy Summit, 700m, DN, 1m, 1, 23.xi.1997, OMNZ., Taieri, DN, 1, ?, NZAC., Outram, Woodside Glen, DN, 1,1♀, 17.xi.1989, LUNZ., Mt Maungatua, High up, DN, 3, ?, NZAC., South bank, Lee Stream, Outram-Hindon Road, pitfall, DN, 7, 1-13.xii.1969, OMNZ(W)., Opoho, Dunedin, DN, 1, 20.viii.1919, AMNZ., Otago, DN, 2, ?.?.1877, BMNH., Otago, DN, 1♂,1♀, ?, BMNH., Otago, DN, 16, 6♂, ii.1984, OMNZ., Robertson Creek, Otago Penninsula, 100m, DN, 3, 8.iv.1994, OMNZ., Pomahake, DN, 1, ?, MONZ., Port Chalmers, DN, 1♀, ?.?.1913, BMNH., Port Chalmers, DN, 3, ?.i.1932, NZAC., Waipori Falls, DN, 2♀,1♂, 18.x.1964, PMJ., Waipori Valley, 140m, DN, 1, 20-22.ii.1998, OMNZ., Waipori, Tuapeka, DN, 1, 2.x.1927, AMNZ., Waitahuna Hill, Meggat Burn, DN, 2m, 1, 5.x.1997, OMNZ., Liberton , DN, 1, 20.iii.1999, OMNZ., Milton, DN, 1, 10.x.1999, OMNZ., Lake Onslow Rd, West of Mt Teviot , DN, 3, 21.ix.1999, OMNZ., Queenstown, OL, 2, 20.iv.1924, AMNZ., Queenstown, OL, 1, 31.xii.1947, AMNZ., Queenstown, OL, 2, 21.11.1946, MONZ., Remarkables, W. face, CO, 4♂,5♀, 27.xi.1981-4.ii.1982, LUNZ., "Clippings" Kingston, Lake Whakatipu, OL, 1♂,1♀, 22.xii.1944, BMNH., Eyre Mts, OL, 1, 5.iv.1915, NZAC., Eyre Mts, Mt Bec, 750m, OL, 1, 23.x.1994, OMNZ., Eyre Mts, Mt Bec, 750m, OL, 2, 3.ii.1968, NZAC., Piano Flat, CO, 1, 22-24.i.1959, NZAC., Clinton, SL, 1♀, 1.viii.1964, PMJ., Clutha Gorge, SL, 2♂,1♀, 14.iv.1968, PMJ., Lumsden, SL, 3♀,2♂, 21.ii.1963, PMJ., Red Duster Ck, Mid Dome, SL, 1♀, 15.ix.1968, NZAC., Catlins, near Owaka, SL, 3, 8.xi.1997, OMNZ., Waikawa, SL, 1♂, 15.xi.1978, CMNZ., Blue Mountains, SL, 1, 13.viii.1969, PMJ., Beaumont, S.F. 60m, SL, 1, 11-20.xii.1995, OMNZ., Wyndham, SL, 2, ?, MONZ., Takitimu Mts, SL, 1, 12.ii.1963, NZAC., Invercargil, SL, 1, ?.x.1919, NZAC.

Description: Medium-sized, 16.5-22.7mm long, shiny black carabid, dorsal surface and legs sometimes red-brown; some populations, e.g., the Rock and Pillar Range, with red femora.
Head: Antennae filiform. Mandibles curved to sharp point, usually bearing single scrobal seta. Labrum shallowly emarginate to non-emarginate, usually with 6 setiferous punctures on apical margin. Lateral depressions of clypeus faint and difficult to discern; frontoclypeal suture always visible. Longitudinal supraorbital depression well defined; 2 supraorbital setae on posterior inner margin and 1-2 (occasionally 3) pairs of setiferous punctures in a shallow transverse depression of the vertex. Frons sometimes transversely wrinkled near apical region of supraorbital depression; otherwise smooth.

Prothorax: Pronotum with margin raised, bearing 8-10 setiferous punctures in no obvious groupings, widest in apical third; disc smooth, in some populations shiny, otherwise matt; median line faintly impressed with anterior and posterior depressions; lateral basal depressions sometimes present though always faint. Mesepisterna distinctly wrinkled.

Abdomen: Elytral striae faintly punctate in some populations, e.g., Central Otago, moderately to distinctly punctate in others, e.g., South Canterbury and Tasman Valley, MK; when present punctations regular, sometimes impressed, which further defines elytral intervals. Elytral margin raised with setiferous punctures in the following groupings, 3-4, 2-3 and 6-12. Ventrites 3-5 with single setae either side of the midline. Ventrite 6 has setae on apical margin, but not either side of the midline as in ventrites 3-5.

Legs: Femora and tibia variable in colour, black-brown in some populations distinctly red in others. The line of spines that run from the apex of the protibia to the base of the antennal cleaning organ is less developed (2-3 spines) than in *O. inaequalis* (5+ spines).

Male genitalia: tip of aedeagus rounded (Figure 2.4A) and generally smaller in diameter than *O. inaequalis*. Left paramere with apical projection of varying length, rarely possesses setae (Figure 2.4E). Ventral surface of right paramere setiferous throughout apical half (Figure 4I). Sclerites X and Y heavily sclerotised, similar to illustrations of *O. inaequalis* (Figure 6A). Apical plate covered in small dark hairs, usually bears 2 apical projections (however a population from the

Hokonui Hills, Southland, is known to have 3); first apical projection heavily sclerotised, second projection (and third when present) weakly sclerotised.

Female genetalia: spermatheca generally short in comparison to *O. septentrionalis* n.sp. and *O. crypticus* n.sp., apical two-thirds maybe distinctly thickened (Figure 2.5E). Spermatheca join bursa copulatrix at small broad flat helminthoid sclerite. Spermatheca sometimes faintly sclerotised, however only visible with staining. Accessory gland elongate, especially in some populations from the Mackenzie Basin. Basal compartment of the accessory gland, sometimes sclerotised, again only visible with staining. Basal segment of female gonocoxite bears 2-3 faintly sclerotised spines on the inner margin (Figure 2.5A).

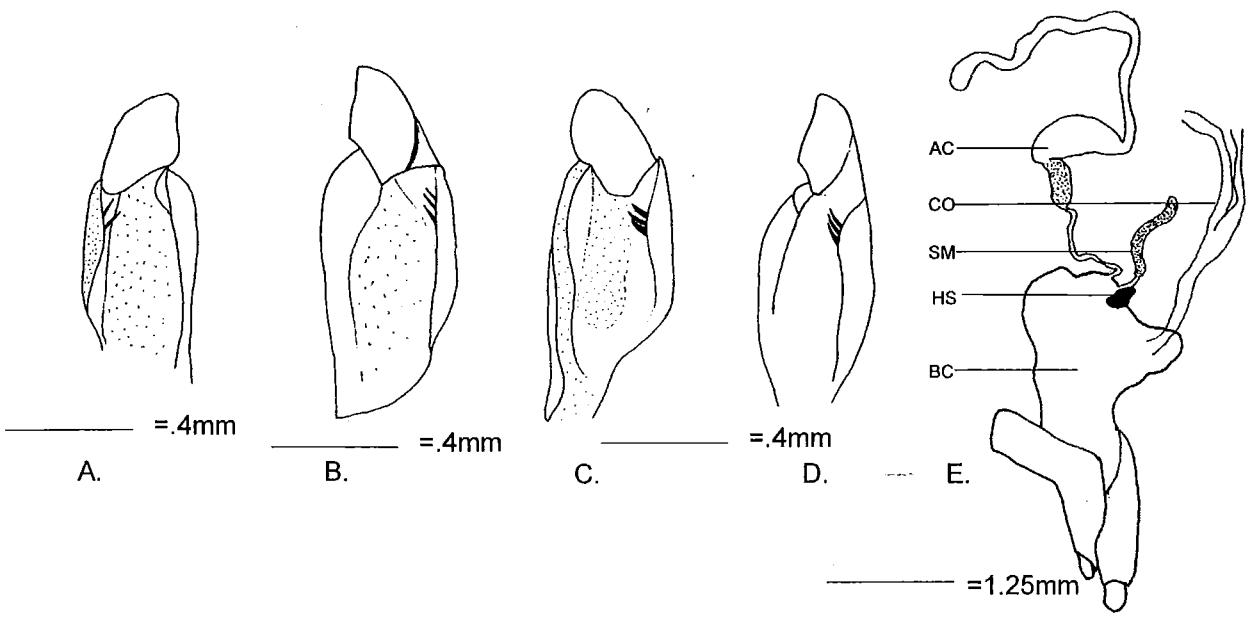


Figure 2.5. A. Right gonocoxite of *O. aereus*, B. Left gonocoxite of *O. inaequalis*, C. Left gonocoxite of O. septentrionalis, D. Left gonocoxite of O. crypticus, E. complete female genitalia of *O. aereus* from Opoho Bush, Dunedin, BC= bursa copulatrix, HS= helmenthoid sclerite, SM= spermatheca, AC= accessory gland, CO= common oviduct.

Distribution: *O. aereus* has been collected from Porters Pass to Invercargill (Figure 2.3). Only two records of *O. aereus* are known to exist from the North Island, one specimen sent by Cpt Thomas Broun to the Museo Civico Di Storia Naturale, G. Doria, Genova, Italy, the other, White's type in The Natural History Museum, London. Though described by White (1846) in, *The Zoology of the Voyage of HMS Erebus and Terror*, the type specimen was, like many other

species described in this publication, not collected as part of the voyage. Accession numbers from the Natural History Museum, London show that it was purchased from Mr Earl who collected widely throughout New Zealand and, at that time, was a resident of Port Nicholson. However, there is nothing associated with the specimen to show it was collected there. The specimen from Broun in Genoa has a number of labels attached to it, none of which are original, including one that reads Nuova Zelanda, Port Nicholson, ex coll. Cap. Th. Broun, 1885: *O. aereus* White, teste Th. Broun (Manual N. 31). One cannot be certain of the exact collection locality due to the lack of original labels and the Wellington label may simply reflect the published distribution at the time of acquisition. Based on such locality information, and the absence of more recent material, it seems unlikely that *O. aereus* was ever present in the Wellington region. Specimens have been collected from a wide variety of habitats including tussock grasslands, shrubland communities, beech forest, broadleaf forest and introduced plantation forests. Generally this species is not associated with W, swampy habitats.

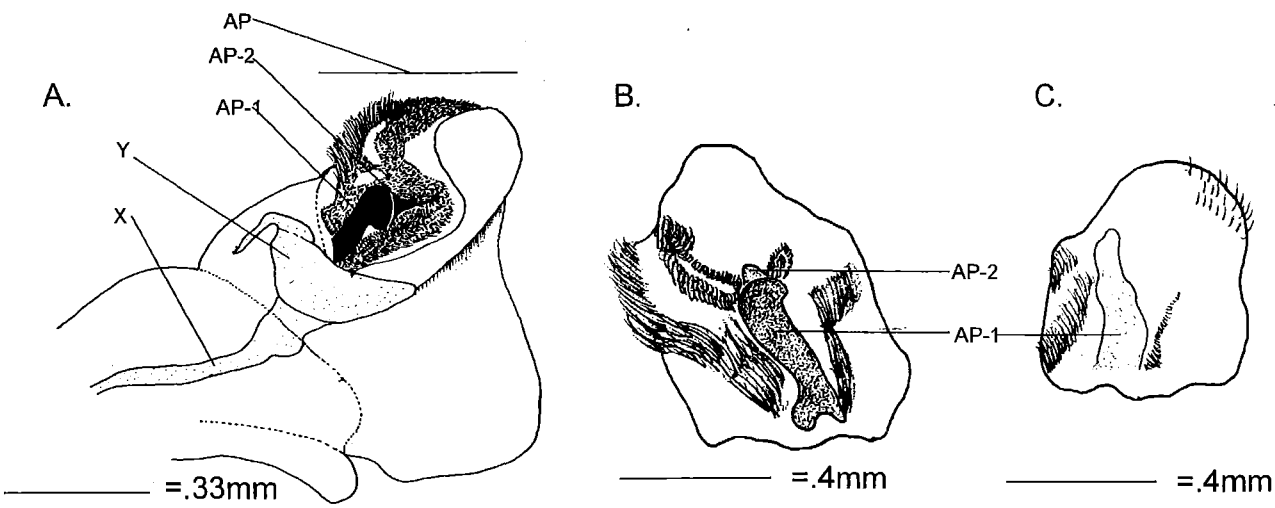


Figure 2.6. A. Male genetalia of *O. inaequalis*, x=sclertie x, y=sclerite y, AP= apical plate, AP-1=first apical projection, AP-2=second apical projection. B. Apical plate of *O. inaequalis*, C. Apical plate of O. septentrionalis .

Oregus inaequalis (Castelnau, 1867)

Original combination: *Mecodema inaequale* Castelnau, 1867:76 (redescribed, 1868:162). Type locality: Dunedin, DN

Type material: No holotype was designated for *O. inaequalis*, however a series of syntypes, Dunedin, 1♂, 3?, -.-.1863, are held at GMI.

Other material examined: 1♀, -.1.1934, BMNH., Bush track Swampy Summit, DN, 1, 26.xi.1993, ITPC., Creek below Burns Saddle, Swampy Summit, DN, 1♂, 2?, 1.xi-11.xii.2000, LUNZ., Dunedin, DN, 1♂, 3?, -.-.1863, GMI Dunedin, DN, 1?, -.v.1908, OMNZ., Flagstaff Hill, DN, 1?, NZAC., Flagstaff Hill, DN, 1♂, NZAC., Harewood, DN, 1?, -.-.1908, OMNZ., Leith Saddle, DN, 1♀, 25.xii.1989, OMNZ., Leith Saddle, DN, 1?, 2.xii.1967, OMNZ(W)., Leith Saddle, DN, 1?, 24.ix.1966, OMNZ(W)., Leith Saddle, DN, 1?, 5.xi.1967, OMNZ(W)., Leith Saddle, DN, 1?, 17.vi.1967, OMNZ(W)., Leith Saddle, DN, 2♀, 6♂, 3.xi-11.xii.200, LUNZ., Leith Valley, DN, 2?, 18.iv.1960, ITPC., Mihiwaka, nr Port Chalmers, DN, 1?, 21/01/47, BMNH., Morrisons Burn, DN, 1?, 12.i.2000, LUNZ., Mt Cargill, DN, 1, 21.x.1981, NZAC., Mt Cargill, DN, 2?, 22.ix.2000, LUNZ., Port Chalmers, DN, 2?, -.ix.1902, MONZ., Port Chalmers, DN, 1?, -.x.1901, BMNH., Port Chalmers, DN, 1?, CMNZ., Ross Ck, DN, 1♂, 12.i.2000, LUNZ., Ross Ck Reservoir, DN, 1?, 18.x.1981, NZAC., Rustlers Ridge, Swampy Summit, DN, 1♂, 1.xi-11.xii.2000, LUNZ., Swampy Summit, DN, 1♂, 1?, 11.ii.1995, OMNZ., Swampy Summit, DN, 2♂, 16.xii.1984-12.i.1985, BIPB., Swampy Summit, DN, 6♂, 17.xi.-16.xii.1984, BIPB., Swampy Summit, DN, 8♂, 3♀, 3?, 24.ix-29.x.2000, LUNZ., Swampy Summit, DN, 7♂, 2♀, 2?, 26.ix-29.x.2000, LUNZ., Swampy Summit, DN, 2?, 29.x.2000, LUNZ., Swampy Summit, DN, 1?, 29.xi.2000, LUNZ., Swampy Summit, DN, 1♀, 23.xi.1997, EEPC., Waitati, DN, 3♀, 1♂ , 3?, 7.x.1923, AMNZ., Waitati, DN, 2?, 7.x.1923, MONZ., Waitati, DN, 1♂, 14.x.1923, AMNZ., Waitati, DN, 1?, 11.xi.1923, AMNZ., Waitati, DN, 1♀, 18.x.1925, AMNZ., Waitati, DN, 4?, 18.ix.1926, AMNZ., Waitati, DN, 2?, 18.ix.1926, BMNH., Waitati Hills, DN, 1♀, 12.ix.1926, NZAC.

Description: Medium-sized carabid, 14.7 mm-18.8 mm long, black, shiny, with a faint oily blue aeneous sheen that varies in intensity between individuals.

Head: Antennae nearly moniliform (Figure 2.7D). Mandible curved forming a sharp point, usually with single scrobal seta, a single tooth. Labrum shallowly emarginate, 6 setae along anterior edge. Clypeus often wrinkled, distinct lateral depressions; frontoclypeal suture distinct. Longitudinal supraorbital depression distinct, 2 setiferous punctures at inner posterior edge (supraorbital setae), 2-5 pairs of setiferous punctures contained in a broad shallow transverse depression of the vertex (Figure 2.7A). Frons smooth, apart from a series of up to 4 distinct wrinkles running parallel and transverse to anterior portion of supraorbital depression. Gula usually with faintly impressed transverse lines; gula suture faintly impressed.

Prothorax: Pronotal margin raised, bearing 10-13 setiferous punctures in no obvious groupings; disc smooth, except for faint lateral basal depressions and occasional short longitudinal wrinkles at anterior margin; median line distinctly impressed. Proepisterna wrinkled in posterior ventral. Mesepisterna highly sculptured, sometimes with distinct longitudinal ridge. Mesosternum, metasternum, metepisterna and metepimera with light transverse wrinkles present in lateral regions.

Abdomen: Ventrites 3-5 have single setae on either side of mid-point, ventrite 6 has 3-5 setiferous punctures on apical margin. Faint transverse wrinkles in lateral regions of all ventrites. Elytral margin raised, setiferous punctures in groups of 6, 3-4, 7-9; striae 1-7 well-defined, deeply impressed and punctate, striae 8-10 poorly defined. Elytral intervals irregular in apical 1/3.

Legs: Outer lateral surface of protibia with distinctive line of spines extending from apex to the base of the antennal cleaning organ. Tarsomeres considerably shorter than in *O. aereus*, *O. septentrionalis*, or *O. crypticus.*

Male genitalia: apex of aedeagus broad, with slight notch in the ventral surface close to the apex (Figure 2.4B). Left paramere with distinct apical projection bearing a number of setae (Figure 2.4F); apical two-thirds of right paramere profusely adorned with long hairs (Figure 2.4J). Internal sac of aedeagus contains sclerites X, Y and an apical plate covered in long dark hairs bearing a large and small sclerotised projection (Figures 2.6A and B); larger projection spatulate and more heavily sclerotised than smaller distal projection; apex of the larger projection distinctly extended to right (Figures 2.6A and B).

Female genetalia: spermatheca relatively short, unsclerotised, joins bursa copulatrix between common oviduct and accessory gland at a small heavily sclerotised helminthoid sclerite. Accessory gland similar in shape to *O. aereus* (Figure 2.5E), but not sclerotised. Gonocoxites bear 2-3 small spines on inner margin (Figure 2.5B).

Distribution: *O. inaequalis* has a very restricted distribution (Figure 2.3). Collected from the Dunedin coastal area it appears restricted to the damp kaikawaka forest and W shrubland/tussock communities of Swampy Summit and surrounding areas.

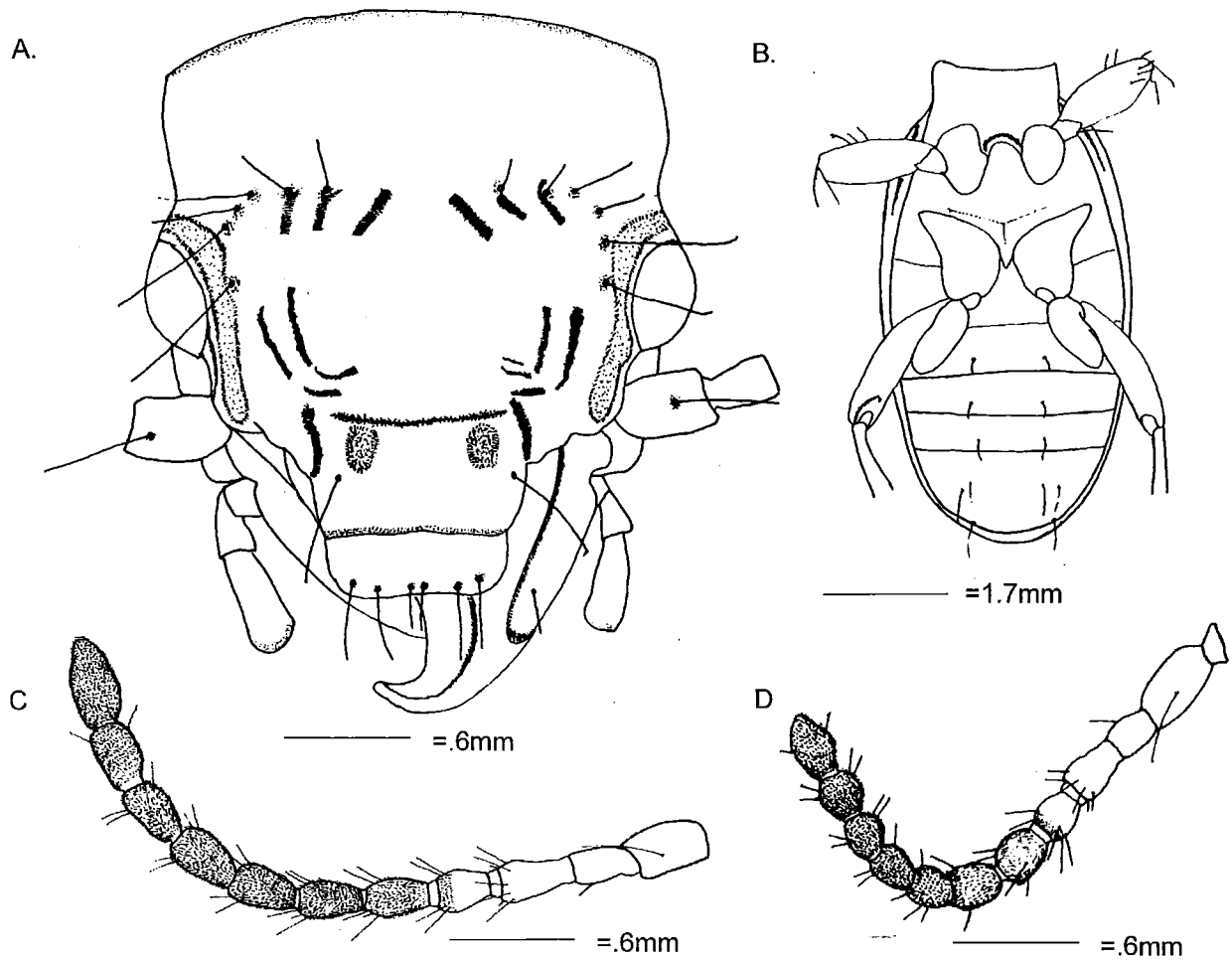


Figure 2.7. A. *O. inaequalis* head, B. Ventral view of the abdomen of O. septentrionalis, C. Filliform antennae of *O. aereus*, D. Moniliform antennae of *O. inaequalis*.

Oregus crypticus Pawson, sp. nov.

HOLOTYPE: Woodend ♂ ?.x.1924 C.E. Clarke, AMNZ.

Material Examined:

Culverden,NC, 1♂, 6.ii.1978, CMNZ., Below Mt Noble, Hurunui Valley, NC, 1♂, 24.iv.1966, PMJ., Upper Hurunui Gorge, NC, 1♂, 20.iv.1962, PMJ., Oxford, NC, 1♀, ?, CMNZ., Woodend, NC, 1♂, ?.x.1924, AMNZ., Eyrewell Forest, NC, 28.i.2001, 3? E.G. Brockerhoff, Eyrewell Reserve, NC, 28.i.2001, 1?, E.G. Brockerhoff, Eyrewell Forest, NC, 29.xii.2000, 3? E.G.Bockerhoff.

DESCRIPTION: Medium-sized carabid, 19.5-20.6 mm long, shiny black dorsal surface, red-brown ventral surface and vividly red/brown legs.

Head: Antennae as in *O. aereus*. Mandibles curved to a sharp point, sometimes with scrobal seta. Labrum shallowly emarginate, 6 setiferous punctures on apical margin; clypeus, broad shallow transverse depression anterior to well-defined frontoclypeal suture. Two supraorbital setae present adjacent to distinct longitudinal supraorbital depression; vertex smooth (in comparison with *O. inaequalis*), transverse depression containing 1-2 pairs of setiferous punctures. Gula with very faint transverse wrinkles.

Prothorax: Pronotal margin raised, with 7-8 setiferous punctures in no obvious groupings; disc smooth except for distinctly impressed midline with anterior and posterior depressions; lateral basal depressions faint to non-existent. Ventral posterior regions of proepisterna faintly wrinkled (much less than *O. inaequalis*); some longitudinal wrinkling of proepimera. Mesepisterna distinctly wrinkled; meso and metasterna smooth but occasionally with faint lateral wrinkling. Metepisterna with a longitudinal ridge that varies in distinctiveness.

Abdomen: Ventrites 3-5 with single setae on either side of the mid-point; ventrite 6 has 1-2 setiferous punctures on apical margin and a pair of central setae either side of the midline (Figure 2.7B). Elytral margin raised with setiferous punctures in the following groupings 4-8,1-3 and 6-7; elytral striae faintly impressed, punctate and regular throughout their length, striae 1-7 more prominent almost reaching elytral apex; intervals 8-11 difficult to distinguish.

Legs: Femora and tibia vivid red/red-brown in colour. The line of spines on the protibia same as an *O. aereus*.

Male genitalia: tip of aedeagus distinctly enlarged with small notch in ventral surface (Figure 2.4C). Left paramere with small non-sclerotised apical projection bearing long setae, setae also present on dorsal margin (Figure 2.4G). Right paramere bares a profusion of long setae along entire ventral margin (Figure 2.4K). Internal sclerites X and Y are present, as described for *Oregus*, though less sclerotised than in *O. inaequalis* or *O. aereus*. Apical plate bears single long straight sclerotised projection, sparsely covered with lightly coloured hairs.

Female genetalia; spermatheca long, thin, unsclerotised, joins bursa copulatrix adjacent to the helminthoid sclerite. Accessory gland located on opposite side of spermathecal duct to the junction of common oviduct and bursacopulatrix. Basal segment of gonacoxite with 3 small spines on inner margin (Figure 2.5C).

Distribution: Only known from a limited number of specimens, this species is restricted to North Canterbury (Figure 2.3). Little is known about its habitat preferences, however some specimens

have been collected from broadleaf shrub tussock communities and recently from introduced pine plantations in Eyrewell Forest.

Etymology: Named after the cryptic nature of its most identifiable character, the enlarged tip of the aedeagus that is internal in repose.

Oregus septentrionalis Pawson, sp. nov.

HOLOTYPE: ♂Hanmer State Forest Park, 7.viii.1985, P. Syrett and H. Harman.

Material Examined:

Hanmer State Forest, 19?, MB, 3.xii.1987, LUNZ., Mt Percival, 3?, KA, 28.x.1962, NZAC., Mt Percival, 1♂, 1?, KA, 29.x.1962, CMNZ., Seaward Kaikoura Range 1?, KA, 13.xii.1993, OMNZ, Sedgemere, 2♂, 2?, MB, 6.ix.1966, , NZAC., Jollies Pass, Hanmer, 3♂,1♀, MB, 29.x.1962, CMNZ., Hanmer State Fore st, 1?, MB, 8.xi.1985, LUNZ., Hanmer State Forest, 3?, MB, 7.viii.1985, LUNZ., Hanmer State Forest, 1?, MB, 5.iv.1978, CMNZ., Hanmer , 1?, MB, 24.ix.1977, LUNZ., Deep Creek, Waiau River, 1♂ , NC, 14.viii.1962, PMJ., Deep Creek, Waiau River, 1♀, NC, 7.viii.1962, PMJ.

Description: Medium-sized, 16.4-18.4 mm long, carabid shiny black/brown dorsal surface with red-brown ventral surface and legs.

Head: Antennae as in *O. aereus*. Mandibles curved with scrobal seta. Vertex, frons and clypeus generally smooth (more so than other species), convex in outline such that longitudinal supraorbital depression is shallow and less obvious than other species. Two supraorbital punctures and 1-2 setiferous punctures located in broad shallow transverse depression of the vertex. Labrum with 6 setiferous punctures on a very shallowly emarginate apical margin. Lateral depressions of clypeus faint, in many cases not present. Frontoclypeal suture defined, but not impressed. Gena and gula both smooth.

Prothorax: Pronotal margin slightly raised, bearing 6-8 setiferous punctures in no obvious groupings; disc smooth, mid line well defined, impressed; basal lateral depressions very faint. Ventroposterior regions of proepisterna faintly wrinkled; distinct longitudinal wrinkling of proepimera. Mesepisterna distinctly wrinkled.

Abdomen: Ventrites 3-5 with 1 setiferous puncture either side of midline; ventrite 6 with 1-3 setiferous punctures either side of midline and 1 or 2 setiferous punctures either side of the midline on the apical margin. Elytral striae faintly impressed, punctate; intervals 1-7 well-defined, intervals 8-11 difficult to distinguish.

Legs: Red-brown in colour.

Male genitalia: left paramere with short non-sclerotised apical projection; some specimens with a group of 4 setae positioned on the inner margin at the base of the apical projection (Figure 2.4H). Tip of aedeagus flattened (Figure 2.4D). Right paramere profusely setose for apical three-quarters of its length (Figure 2.4L). Apical plate with few short hairs and a single weakly sclerotised projection (Figure 2.6C).

Female genetalia: basal segment of gonocoxite with 3 small spines on inner margin (Figure 2.5D); spermatheca long, thin, joining bursa copulatrix at a small heavily sclerotised helminthoid sclerite, located between common oviduct and accessory gland. Accessory gland with distinctly ribbed section prior to elongate apical portion.

Distribution: *O. septentrionalis* is the most northern species of *Oregus* (Figure 2.3), known from the Waiau Valley, NC. and several localities in Marlborough. It has been collected from relatively dry beech forest remnants as well as introduced pine plantations.

Etymology: The name indicates its relatively northern distribution.

Chapter 3 Molecular Systematics of the Genus *Oregus*

Introduction

The use of molecular techniques such as DNA sequencing for phylogenetic studies has rapidly gained momentum in the past decade. This is reflected in the large number of phylogenetic papers that have a molecular component (Caterino *et al.*, 2000), and the proportion of papers in journals such as Systematic Entomology and Systematic Biology that utilise molecular techniques. DNA sequencing was seen as an appropriate method to test morphological species designations as *Oregus* is morphologically relatively conservative. DNA sequencing provides a means to analyse the extent of genetic diversity between populations of *Oregus* that have a few morphological differences. This genetic diversity can then be compared with that between the currently designated species.

The cytochrome oxidase I (COI) gene region has been used extensively for studying the phylogeny of various groups of Coleoptera, including Carabidae (Galian *et al.*, 1999), Dytiscidae (Ribera *et al.*,), Curculionidae (Langor and Sperling, 1997; Kelly *et al.*, 1999; Sequeira *et al.*, 2000), Tenebrionidae (Juan *et al.*, 1996a; 1996b), Chyrsomelidae (Funk, 1999) and a general phylogeny of 15 coleopteran families (Howland and Hewitt, 1995). The COI gene region was chosen for this study for three reasons. First, the general properties of mtDNA (refer to chapter 1), second, COI is the most commonly sequenced gene region in studies of insect systematics (Caterino *et al.*, 2000) and third COI is known to evolve at a rate sufficient to examine phylogenies at the species/population level (Simon *et al.*, 1994).

The NADH dehydrogenase subunit 1 (ND1) gene region was chosen for its relatively rapid rate of sequence evolution, sufficient to examine inter-population variability (Simon, 1991). ND1 has, in comparison to other regions (e.g., COI and COII and Cytochrome B), been utilised less frequently in phylogenetic studies (Caterino *et al.*, 2000). A search of Genbank produced four studies (two published) that used the ND1 gene region for carabids. The study by Prüser and Mossakowski (1998) indicated intraspecific sequence divergence of 0.57-4.0%, sufficient to examine phylogenetic structure between populations of a single species and closely related species.

Düering and Brückner (2000) utilised ND1 to determine the species/genera relationships within the tribe Molopini.

There are two main objectives for the molecular study of the genus *Oregus*: (i) to resolve the phylogeny of the group, and establish if *O. aereus* is a single species or a complex of several species as suggested by Jamieson (1999) and (ii) to provide an independent data set to compare the results of the morphological hypothesis of phylogeny developed in chapter 2 of this study which suggests that the genus consists of four species.

Material and Methods

Collections

All specimens were collected by hand and placed directly into 96% ethanol and then stored at 4°C to prevent degradation of the DNA prior to extraction.

DNA Extraction

The prothorax and associated prothoracic legs of each specimen were excised and homogenised in 250µl digestion buffer for 2-3 hours @ 50°C. The digestion buffer comprised 25µl SET (0.1M NaCl, 1mM EDTA, pH 8.0, 10mM Tris), 25µl 10% SDS pH 7.2, 20µl of 10mg/ml Proteinase-K and 180µl dIH_2O. Total DNA was extracted using a silica-based DNA purification matrix (Prep-A-Gene® Bio-Rad) following the manufacturers instructions. The Prep-A-Gene matrix was used for its ability to extract high-quality DNA, while removing unwanted proteins, pigments and other inhibitory compounds. The total genomic DNA extracted was resuspended in 50µl of TE, pH 8.0 and stored at 4°C.

DNA Amplification and Sequencing

The PCR was carried out in 10µl volumes containing 0.11µl of 3.5u/µl Expand™ High Fidelity *Taq* polymerase (Roche), 1µl 10x Expand™ High Fidelity buffer with $MgCl_2$ (1.5µM) (Roche), 1.5µl 10x dNTPs, 1.2µl 2mM each primer, 0.4µl genomic DNA and 4.59µl dIH_2O. The sequence and position of primers is provided in Figure 3.1. A Gene Amp® PCR System 2400 (Perkin-Elmer) thermal cycler was used with the following temperature profile, 94°C for 2 min denaturation followed by 38-40 cycles of 94°C for 15 sec, 50°C for 30 sec, 72°C for 1 min and a final extension of 72°C for 2 min. The quality and quantity of the PCR products were assessed using submerged gel electrophoresis (4.84V/cm, 500mA, 1% agarose gel, ethidium bromide stained,

visualised with UV light, run with XIV ladder (ROCHE) and quantified with low mass ladder (Gibbco, BRL)) for 30 minutes.

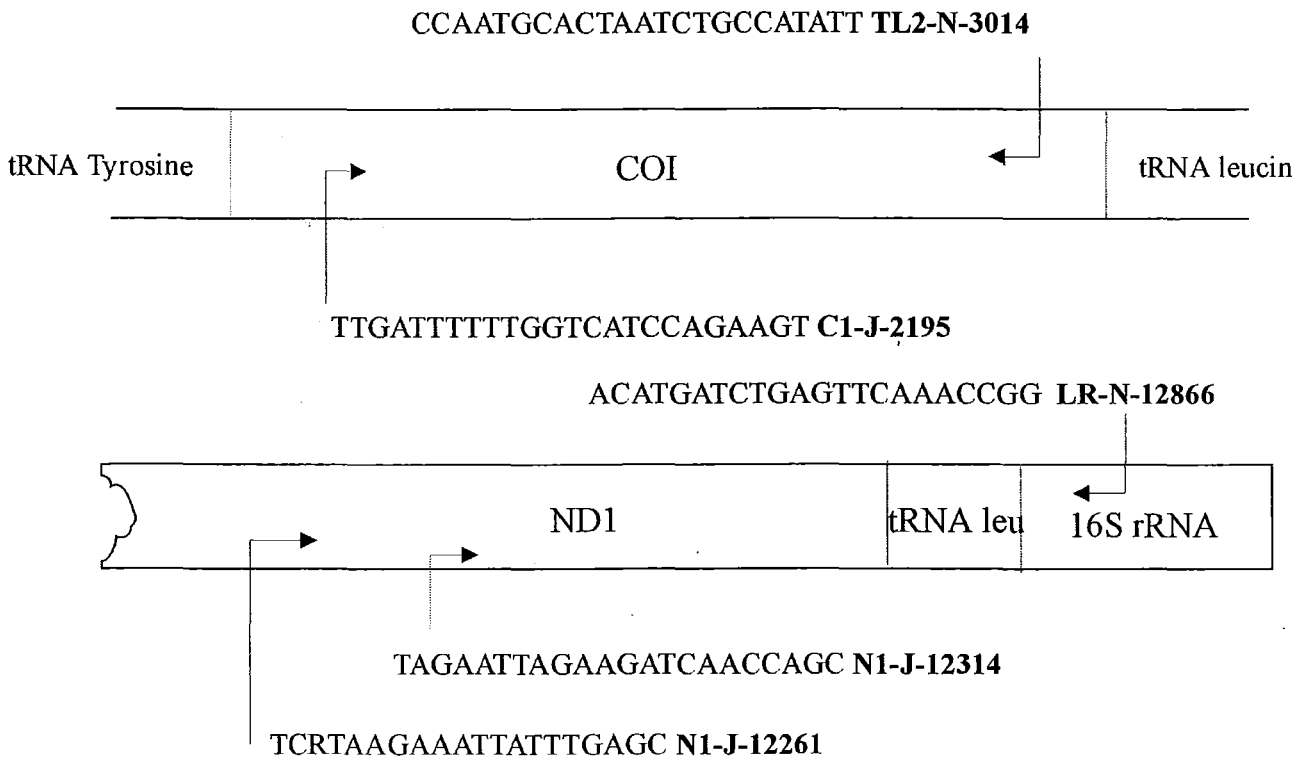


Figure 3.1. Primers used to PCR amplify the COI and ND1 gene regions, the nomenclature follows that of Simon *et al.* (1994).

Excess salts, primers and dNTPs were removed from the PCR product by isopropanol precipitation (32µl) in the presence of 0.67M NH_4Ac followed by a wash in 70% EtOH. The products were resuspended and 6.2µl dIH_2O and quantified by submerged gel electrophoresis (80V, 500 mA) for 30 minutes. Sequencing reactions (10µl) were carried out using 0.8µl 2mM primer, 2.6µl PCR product, 2.6µl dIH_2O and 4 µl ABI PRISM® Big Dye™ (Applied Biosystemsand following the manufacturers protocol. Sequence products were then purified by ethanol precipitation, air-dried and sent to the University of Waikato for reading in a ABI-PRISM® 377 automatic sequencer.

Data Analysis

Forward and reverse sequences were compared using Sequencher™ (Gene Codes: Version 4.0.5) to correct for sequence ambiguities. Sequences were then aligned using the multiple alignment function of Clustal-X. (Thompson *et al.*, 1997). Analysis was performed in PAUP V4.0b8 (Swofford, 1998).

Model Test V3.06 (Posada and Crandall, 1998) was used to determine which model of sequence evolution best described the data sets. These models were COI=TVM+G, ND1=TIM and for the combined data TVM+G. The substitution rate matrix and gama distribution shape parameters used are outlined in Table 3.2. Additional sequence replicates were toggled as-is, neither the molecular clock nor steepest descent option were enforced.

Maximum parsimony analyses assumed a matrix of unordered characters (Fitch, 1971a) with equal phylogenetic weighting of all sites. Each data set was analysed separately to determine the most likely and most parsimonious tree. The associated consistency index (Kluge and Farris, 1969) and retention index (Farris, 1989) of each tree was determined to assess levels of homoplasy. The G_1-statistic (Hillis, 1991; Hillis and Huelsenbeck, 1992; Huelsenbeck, 1991) was used to determine the strength of the phylogenetic signal. A combined analysis of the two gene regions was conducted conditional to a length incongruence test (partition homogeneity test) (Farris *et al.*, 1995).

Results

South Island collection localities of specimens used in the molecular analysis are shown in figure 3.2 and table 3.1. The substitution rate matrices used for the maximum likelihood analysis were generated with model test and the values are shown in table 3.2 with the empirical nucleotide base frequencies in table 3.3.

All populations were successfully sequenced and both mtDNA loci included in the analysis with the exception of two specimens of *O. aereus* from the Pisa Range (Central Otago). COI Sequences from these specimens corresponded well with other populations of *O. aereus* from geographically adjacent areas. However, the sequences for ND1 showed significant departures from other populations (Appendix F) and appeared to be of a different size (Figure 3.3). The se-

quences did not appear to be insect mtDNA as they lacked the frequently reported A/T richness (Langor and Sperling, 1997, Funk, 1999). Sequences from the Pisa Range had a G/C content of 16/23%, whereas on average the other 23 individuals of *Oregus* had a G/C content of 10/9%. Sequences from both specimens were read clearly and were identical apart from two single base pair substitutions. They were therefore considered to be authentic amplifications with primers N1-J-12886 and N1-J-12261, and not an error in the sequencing process. To investigate the origin of the sequences a Genbank Blast search (Altschul *et al.*, 1997) was performed. This was uninformative as the closest match was 40 base pairs out of 444, compared to a similar Blast search with the other ND1 sequences that successfully showed close sequence matches with other carabids from a study by Pruser and Mossakowski (1998) and by a German researcher Andreas Düering (Tribe Molopini) (unpublished data). Therefore, it is known that the other 23 sequences represent true 16S rRNA, $tRNA_{leu}$ and ND1 coding regions. Nucleotide sequences from the Pisa Range were translated to the amino acid sequence, but each of the three reading frames resulted in stop codons throughout what should have been the ND1 coding region. Therefore, it is assumed that the sequence represents a noncoding piece of DNA. A possible conclusion is a mitochondrial insertion of the nucleus (pseudogene/numt) that has undergone random mutation (Bensasson *et al.*, 2001; Zhang and Hewitt, 1996b). However, uncorrected sequence divergence of the Pisa Range population is 65% compared with other individuals from the genus *Oregus*. This is inconsistent with previous identified pseudogenes that have sequence divergence of 1-25% (Bensasson *et al.*, 2001). Such high sequence divergence suggests that the observed anomalous sequences are not mitochondrial pseudogenes. Close examination of the PCR products on agarose gels shows that bands from the Pisa Range population are slightly smaller in size (Figure 3.3) than other populations of *O. aereus*. This combined with a relatively low annealing temperature (52°C, which was an improvement on previous published annealing temperatures for this primer of 47-50°C (Hedin, 1997)) suggests a spurious non-target PCR product, possibly of nuclear origin given the GC content. Therefore, in this study ND1 sequences from the Pisa Range were excluded from the ND1 and combined analysis to prevent bias in the molecular phylogeny. It was interesting that these anomalous sequences were only found in two specimens collected from a particular population and implies a consistent polymorphism in the priming site region within ND1. Future attempts to amplify the correct ND1 sequence for the Pisa Range specimens could purify the mtDNA or utilise different primers as suggested by Bensasson *et al.* (2001).

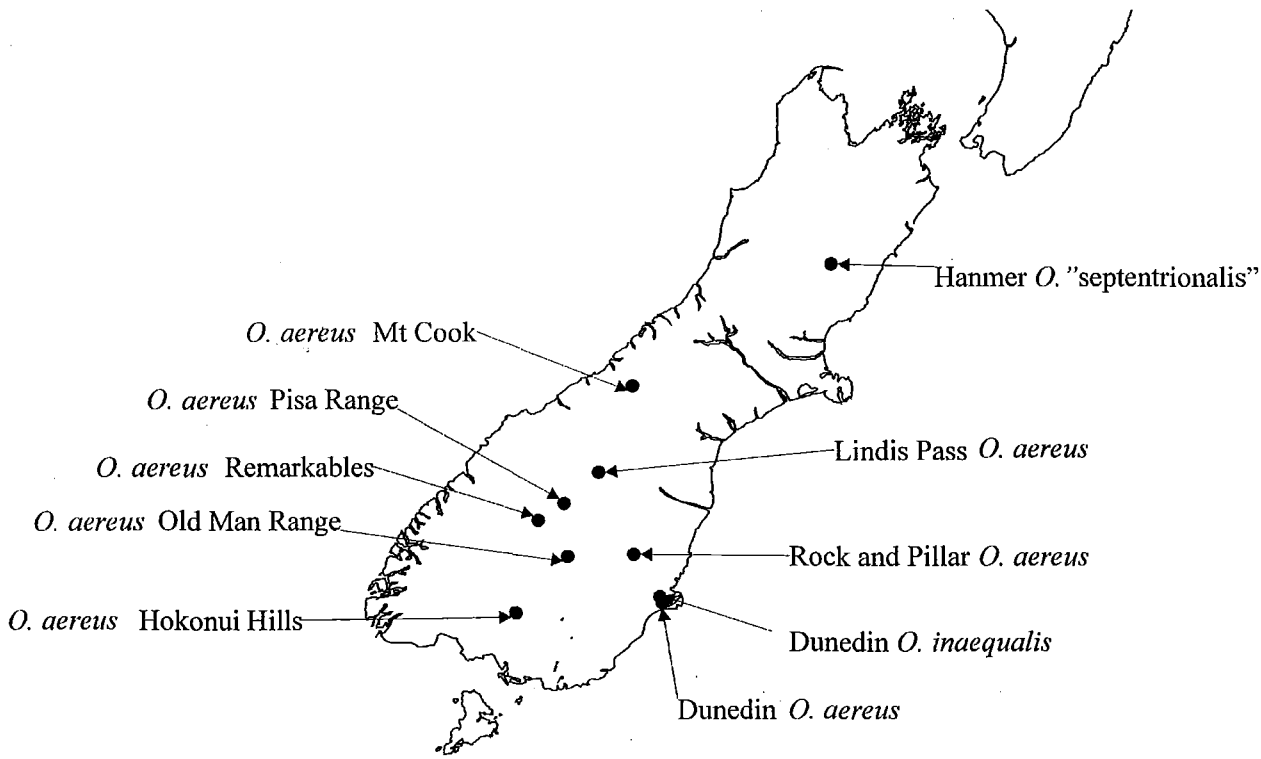


Figure 3.2. Location of specimens collected for sequencing

Table 3.1 Collection localities and Genbank accession numbers.

Species (Population)	Locality and Collector	Genbank accession COI	Genbank Accession ND1
Oregus inaequalis 1	Swampy Summit, Dunedin-Sept 2000, S.M. Pawson	AF466849	AF466864
Oregus inaequalis 2	Swampy Summit, Dunedin-Sept 2000, S.M. Pawson	AF466850	AF466865
Oregus inaequalis 3	Swampy Summit, Dunedin-Sept 2000, S.M. Pawson	AF466851	AF466866
Oregus inaequalis 4	Swampy Summit, Dunedin-Sept 2000, S.M. Pawson	AF466852	AF466867
Oregus aereus (Dunedin) 1	Woodhaugh Park, Dunedin, 10.i.2001, S.M. Pawson and K.B.R Hill	AF466834	AF466870
Oregus aereus (Dunedin) 2	Chalkies Creek, Nr Dunedin, 10.i.2001, S.M. Pawson and K.B.R Hill	AF466835	AF466856
Oregus aereus (Dunedin) 3	Ross Creek, Dunedin 10.i.2001, S.M. Pawson and K.B.R Hill	AF466836	AF466857
Oregus aereus (Dunedin) 4	Chalkies Creek, Nr Dunedin, 10.i.2001, S.M. Pawson and K.B.R Hill	AF466845	AF466858
Oregus aereus (Rock and Pillar) 1	Johnson Rd, Shannon, 19.ii.2001, S.M. Pawson	AF466846	AF466855
Oregus aereus (Rock and Pillar) 2	Johnson Rd, Shannon, 19.ii.2001, S.M. Pawson	AF466853	AF466872
Oregus aereus (Mt Cook) 1	Bush Stream, Tasman Valley, 26.ii.2001, S.M. Pawson	AF466837	AF466859
Oregus aereus (Mt Cook) 2	Bush Stream, Tasman Valley, 26.ii.2001, S.M. Pawson	AF466838	AF466860
Oregus aereus (Old Man Range) 1	Omeo Gully, Old Man Range, 15.x.2001, S.M.Pawson	AF466841	*AF466874
Oregus aereus (Old Man Range) 2	Omeo Gully, Old Man Range, 15.x.2001, S.M.Pawson	AF466843	*AF466868
Oregus aereus (Hokonui Hills)	Mt Peel, Hokonui Hills, 18.x.2001, S.M. Pawson	AF466839	*AF466871
Oregus aereus (Remarkables)	Remarkables, 18km N of Kingston, 19.x.2001, S.M. Pawson	AF466840	*AF466873
Oregus aereus (Pisa Range) 1	Mt Pisa Station, 900m, 19.x.2001, S.M. Pawson	AF466842	*Not Submitted
Oregus aereus (Pisa Range) 2	Mt Pisa Station, 900m, 19.x.2001, S.M. Pawson	AF466844	*Not Submitted
Oregus aereus (Lindis Pass)	Below Breast Hill, Lindis Pass, 14.x.2001, S.M. Pawson	AF466833	*AF466869
Oregus septentrionalis 1	Hanmer Nature Walk, 4.i.2001, S.M. Pawson and K.B.R Hill	AF466847	AF466861
Oregus septentrionalis 2	Hanmer Nature Walk, 4.i.2001, S.M. Pawson and K.B.R Hill	AF466848	AF466862
Oregus septentrionalis 3	Hanmer Nature Walk, 4.i.2001, S.M. Pawson and K.B.R Hill	AF466854	AF466863
Diglymma clivinoides 1	Lake Sylvestor Track, Cobb Reservoir 1.ii.2001, S.M. Pawson and K.B.R Hill	AF466830	AF466875
Diglymma clivinoides 2	Lake Sylvestor Track, Cobb Reservoir 1.ii.2001, S.M. Pawson and K.B.R Hill	AF466831	AF466876
Diglymma clivinoides 3	Lake Sylvestor Track, Cobb Reservoir 1.ii.2001, S.M. Pawson and K.B.R Hill	AF466832	AF466877

Specimens marked with an * were amplified using primer N1-J-12261 not N1-J-12314.

Table 3.2. Substitution rate matrix for maximum likelihood analysis. Models estimated using Model Test V3.06 (Posada and Crandall, 1998)

Gene region	Substitution model	A-C	A-G	A-T	C-G	C-T	G-T	Gama shape parameter	Proportion Invariable site
COI	TVM+G	8.8163	13.9811	16.2748	0.0000000001	88.2411	1	Equal Rates	0.7681
ND1	TIM	1	1470991.3750	506603.652	506603.652	1470991.375	1	0.178	0
Combined	TVM+G	6.1774	52.2494	27.8161	1.2920	52.2494	1	0.1319	0

Table 3.3 Empirical nucleotide base frequencies, values in parenthesis are averages across all sequences.

Codon Position	Base Composition				Percent variable sites
	A	C	G	T	
COI pos. 1	29.8-33.5 (30.5)	10.1-12.1 (11.3)	25.8-26.6 (26.3)	29.8-33.5 (31.9)	10.90
ND1 pos.1	29.1-29.1 (29.1)	11.7-11.7 (11.7)	15.5-15.5 (15.5)	42.7-43.7 (42.8)	6.80
COI pos.2	17.5-18.7 (18.7)	23.6-24.4 (23.6)	15.0-15.9 (15.8)	41.9-42.3 (41.9)	1.60
ND1 pos.2	22.3-22.3 (22.3)	13.6-16.5 (14.7)	11.7-11.7 (11.7)	49.5-52.4 (51.3)	4.90
COI pos.3	46.2-49.0 (48.0)	2.8-4.5(3.7)	0.4-2.0 (0.9)	46.6-48.6 (47.3)	33.60
ND1 pos.3	37.3-42.2 (38.8)	1.o-2.9 (1.8)	1.0-5.9 (4.1)	52.9-56.9 (55.3)	36.39
COI all sites	31.5-32.9 (32.4)	12.5-13.1 (12.8)	14.2-14.8 (14.3)	39.8-40.7 (40.3)	15.40
ND1all sites	29.5-31.2 (30.1)	9.1-9.7 (9.4)	9.4-11.0 (10.4)	49.0-50.3 (49.8)	16.07

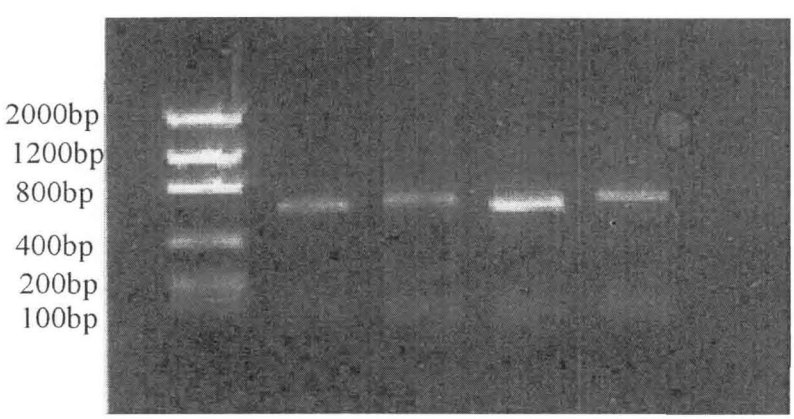


Figure 3.3. ND1 PCR product amplified using primers N1-J-12261 and LR-N-12866, run on a 1% agarose gel, 4.84V/cm, 500mA for 30min. A. *O. aereus*, Pisa Range 1; B. *O. aereus*, Old Man Range; C. *O. aereus*, Pisa Range 2; D. *O. aereus*, Lindis Pass.

Table 3.4. Corrected sequence divergence (HKY-85 (Hasegawa, 1985)) as a percentage, COI lower left half of table and ND1 upper right.

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	*Oregus aereus*	Old Man Range	-	0.00	1.63	1.63	1.16	1.87	1.87	1.87	1.87	1.63	1.63	1.87	1.87			3.05	3.05	3.05	2.80	3.28	3.28	3.28	9.59	9.59	9.59
2	*Oregus aereus*	Old Man Range	0.00	-	1.62	1.62	1.15	1.86	1.86	1.86	1.86	1.62	1.62	1.86	1.86			3.03	3.03	3.03	2.79	3.27	3.27	3.27	10.07	10.07	10.07
3	*Oregus aereus*	Mt Cook	2.34	2.34	-	0.00	0.46	1.63	1.63	1.63	1.63	1.39	1.40	1.63	1.63			3.54	3.54	3.54	3.05	4.03	4.03	4.02	9.32	9.32	9.32
4	*Oregus aereus*	Mt Cook	2.20	2.20	0.14	-	0.46	1.63	1.63	1.63	1.63	1.39	1.40	1.63	1.63			3.54	3.54	3.54	3.05	4.03	4.03	4.02	9.32	9.32	9.32
5	*Oregus aereus*	Lindis Pass	2.48	2.48	0.41	0.27	-	1.16	1.16	1.16	1.16	0.92	0.92	1.16	1.16			3.05	3.05	3.05	2.56	3.53	3.53	3.52	8.78	8.78	8.78
6	*Oregus aereus*	Dunedin 2	1.64	1.64	1.23	1.09	1.37	-	0.00	0.00	0.00	1.15	1.15	0.69	0.69			3.55	3.55	3.55	3.06	4.03	4.03	4.03	9.85	9.85	9.85
7	*Oregus aereus*	Dunedin 4	1.78	1.78	1.37	1.23	1.51	0.14	-	0.00	0.00	1.15	1.15	0.69	0.69			3.55	3.55	3.55	3.06	4.03	4.03	4.03	9.85	9.85	9.85
8	*Oregus aereus*	Dunedin 1	1.64	1.64	1.23	1.09	1.37	0.00	0.14	-	0.00	1.15	1.15	0.69	0.69			3.55	3.55	3.55	3.06	4.04	4.04	4.03	9.86	9.86	9.86
9	*Oregus aereus*	Dunedin 3	1.64	1.64	1.23	1.09	1.37	0.00	0.14	0.00	-	1.15	1.15	0.69	0.69			3.55	3.55	3.55	3.06	4.04	4.04	4.03	9.86	9.86	9.86
10	*Oregus aereus*	Hokonui Hills	1.64	1.64	1.23	1.09	1.37	0.27	0.41	0.27	0.27	-	0.00	0.69	0.69			3.53	3.53	3.53	3.04	4.01	4.01	4.01	9.32	9.32	9.32
11	*Oregus aereus*	Remarkables	1.78	1.78	1.37	1.23	1.51	0.41	0.54	0.41	0.41	0.14	-	0.69	0.69			3.54	3.54	3.54	3.05	4.02	4.02	4.02	9.32	9.32	9.32
12	*Oregus aereus*	Rock and Pillar	1.78	1.78	1.09	0.95	1.23	0.41	0.54	0.41	0.41	0.41	0.54	-	0.00			3.31	3.31	3.31	2.82	3.79	3.79	3.78	9.59	9.59	9.59
13	*Oregus aereus*	Rock and Pillar	1.78	1.78	1.09	0.95	1.23	0.41	0.54	0.41	0.41	0.41	0.54	0.00	-			3.31	3.31	3.31	2.82	3.79	3.79	3.78	9.59	9.59	9.59
14	*Oregus aereus*	Pisa Range	1.50	1.50	1.09	0.95	1.23	0.41	0.54	0.41	0.41	0.41	0.54	0.27	0.27	-		Sequence not obtained									
15	*Oregus aereus*	Pisa Range	1.50	1.50	1.09	0.95	1.23	0.41	0.54	0.41	0.41	0.41	0.54	0.27	0.27	0	-										
16	*Oregus inaequalis*		3.19	3.19	3.05	2.90	3.19	2.90	3.05	2.90	2.90	2.62	2.76	2.76	2.76	2.48	2.48	-	0.00	0.00	0.46	3.80	3.80	3.79	9.86	9.86	9.86
17	*Oregus inaequalis*		3.19	3.19	3.05	2.90	3.19	2.90	3.05	2.90	2.90	2.62	2.76	2.76	2.76	2.48	2.48	0.00	-	0.00	0.46	3.80	3.80	3.79	9.86	9.86	9.86
18	*Oregus inaequalis*		3.19	3.19	3.05	2.90	3.19	2.90	3.05	2.90	2.90	2.62	2.76	2.76	2.76	2.48	2.48	0.00	0.00	-	0.46	3.80	3.80	3.79	9.86	9.86	9.86
19	*Oregus inaequalis*		3.19	3.19	3.05	2.90	3.19	2.90	3.05	2.90	2.90	2.62	2.76	2.76	2.76	2.48	2.48	0.27	0.27	0.27	-	3.30	3.30	3.30	9.32	9.32	9.32
20	*Oregus septentrionalis*		4.92	4.92	5.36	5.21	5.51	4.33	4.48	4.33	4.33	4.32	4.47	4.77	4.77	4.48	4.48	5.20	5.20	5.20	5.20	-	0.00	0.00	9.58	9.58	9.58
21	*Oregus septentrionalis*		4.92	4.92	5.36	5.21	5.51	4.33	4.48	4.33	4.33	4.32	4.47	4.77	4.77	4.48	4.48	5.20	5.20	5.20	5.20	0.00	-	0.00	9.58	9.58	9.58
22	*Oregus septentrionalis*		4.92	4.92	5.36	5.21	5.51	4.33	4.48	4.33	4.33	4.32	4.47	4.77	4.77	4.48	4.48	5.20	5.20	5.20	5.20	0.00	0.00	-	9.57	9.57	9.57
23	*Diglymma clivinoides*		11.58	11.58	12.05	11.89	12.05	10.94	11.10	10.94	10.94	10.93	11.10	11.41	11.41	11.26	11.26	11.90	11.90	11.90	11.74	11.73	11.73	11.73	-	0.00	0.00
24	*Diglymma clivinoides*		11.81	11.81	12.30	12.13	12.30	11.18	11.33	11.18	11.18	11.17	11.34	11.65	11.65	11.49	11.49	12.14	12.14	12.14	11.98	11.97	11.97	11.97	0.14	-	0.00
25	*Diglymma clivinoides*		11.73	11.73	12.21	12.05	12.21	11.10	11.26	11.10	11.10	11.09	11.26	11.57	11.57	11.40	11.40	11.90	11.90	11.90	11.74	11.89	11.89	11.89	0.14	0.00	-

Populations of *O.aereus*
O.inaequalis
O. septentrionalis
D. clivinoides

COI

A 741 base pair segment was amplified in all taxa corresponding to positions 2232-2974 of the *Drosophila yakuba* mitochondrial gene sequence (Clary and Wolstenholme, 1985). The aligned sequences are shown in appendix F. The sequence exhibits typical A/T nucleotide bias (Table 3.3) that is commonly reported for insect COI mtDNA (Lunt *et al.*, 1996; Langor and Sperling, 1997; Funk, 1999). A/T bias was most pronounced at the third codon position, also noted by Brown *et al.* (1994) and Lunt *et al.* (1996). There were 114 (15.3%) variable sites of which 27 (23.5%), 4 (3.5%) and 83 (73%) were at the 1st, 2nd and 3rd codon positions respectively. Corrected sequence divergence (using HKY-85 (Hasegawa *et al.*, 1985)) within the ingroup taxa ranged from 0.0-5.5%. Sequence divergences between outgroup and ingroup taxa ranged between 10.9-12.3%. The population of *O. aereus* from the Old Man Range was the most divergent of all populations sequenced for that taxon (Table 3.4). *O. septentrionalis* was the most divergent taxon of the ingroup taxa, 4.3-5.5% corrected sequence divergence (Table 3.4). A G_1-statistic (Hillis, 1991; Huelsenbeck, 1991; Hillis and Huelsenbeck, 1992) of –1.91 (evaluating 10,000 random trees) indicates high phylogenetic signal/noise ratio.

The single maximum likelihood tree (ln 1669.59825) was less clear in its resolution than the parsimony tree (Appendix B). Specimens of *O. septentrionalis* formed the most basal clade of the ingroup taxa. Although the likelihood tree indicates an alternative placement of *O. inaequalis*, tree-to-tree distances show this to be insignificant. Furthermore this alternative placement appears to be the result of long branch attraction (Figure 3.6, see discussion). Maximum parsimony analysis produced 15 trees of 139 steps, CI= 0.90, RI=0.96 (Appendix C). The ingroup portion of the tree suggests three main divisions. *O. septentrionalis* appears to be the most basal of the ingroup taxa. *O. inaequalis* is separated with good bootstrap support (100%) from a third division that includes all populations of *O. aereus*.

ND1, tRNAleu and 16sRNA

PCR amplification of the ND1 gene region proved difficult for some specimens, producing weak/no products. Alternative annealing temperatures, DNA concentrations and the use of DMSO (di-methyl sulfoxide) were tried to improve yield, however efforts were unsuccessful. The primer N1-J-12314 (used by Düering and Brückner (2000), Pruser and Mossakowski (1998) and Pashley and Ke (1992)) was replaced in seven specimens with N1-J-12261 (used by Hedin

(1997) for spiders, as a colleague was using this primer and it worked for *Oregus*) and used at a higher annealing temperature (52° vs 47°). Improved yields of PCR products were attained more consistently with
N1-J-12261. Due to the mechanism by which PCR works, continued cycling promotes the amplification of products with identical sequences to the primer at the priming site. As such it is difficult to detect sequence variation at priming sites. However, by amplifying sequences using the alternative N1-J-12261 primer that was located outside the original PCR fragment it was possible to accurately assess the nucleotide sequence polymorphism at priming site N1-J-12314. Of the seven sequences amplified using N1-J-12261 four obtained accurate reads for either, part of, or the entire N1-J-12314 priming site. Five base pair changes were identified, four involving T to A substitutions. Such discrepancies are not unexpected given their placement in the ND1 gene region, however it does suggest the need for better primers that are more conserved.

A 444 base pair fragment corresponding to positions 12822-12378 of the *Drosophila yakuba* mitochondrial gene sequence was successfully amplified (Clary and Wolstenholme, 1985). The aligned sequences are shown in appendix F. Sequences included the 16sRNA (partial, bases 1-61), $tRNA_{leu}$ (complete, bases 62-118) and the ND1 gene (partial, 137-444) [two sequences from individuals collected at the Pisa Range were omitted due to the sequencing of an unidentified product, see discussion}. There was distinct A/T nucleotide bias, with increased A/T content at the 3rd position (Table 3.3). There were a total of 49 (16%) variable sites within the ND1 portion of the sequence; 7(14%), 5(10%) and 37(76%) of those changes at the 1st, 2nd and 3rd codon positions respectively. Corrected sequence divergence (of the ND1 portion of the ingroup taxa), using HKY-85 (Hasegawa *et al.*, 1985) ranged from 9.3-10.7% between outgroup and ingroup taxa (Table 3.4). Sequence divergence within the ingroup taxa ranged from 0.0-4.3%. The population of *O. aereus* from the Old Man Range was, as also shown by COI, the most divergent of all populations sequenced for that taxon (Table 3.4). *O. septentrionalis* was the most divergent taxon (as also shown by COI) of the ingroup taxa, 3.2-4.0% (Table 3.4). A G_1-statistic (Hillis, 1991; Huelsenbeck, 1991; Hillis and Huelsenbeck, 1992) of -1.44 indicating strong phylogenetic signal/noise ratio.

Maximum parsimony analysis of the ND1 gene region produced 3 equally parsimonious trees of 72 steps (CI=0.89, RI=0.96) (Appendix E). The topology was identical to the parsimony tree for the COI gene region (Appendix C). Maximum likelihood analysis produced a single tree, ln

906.08 (Appendix D) that was identical to the ND1 parsimony tree (Appendix E), apart from a single collapsed branch leading to the Rock and Pillar taxa. *O. septentrionalis* was the most basal of the ingroup species as for COI and *O. inaequalis* was the sister taxon to the more derived clade that included six populations of *O. aereus*.

Combined COI and ND1 data

An incongruence length difference test (partition homogeneity test in PAUP) (Farris *et al.*, 1995) showed no incongruence (P=1.0) between the CO I and ND1 sequence partitions (the 16S RNA, $tRNA_{leu}$ and associated spacer regions were included as part of the ND1 data partition for this test). The phylogenies for the combined data sets are shown in Figures 3.4 and 3.5. Maximum parsimony analysis produced two equally parsimonious trees of 210 steps (CI = 0.90, RI = 0.96). The only difference between the two trees was the association of the Rock and Pillar population with the Dunedin population in one tree and populations from Hokonui and the Remarkables in the other. These differences correspond to the most parsimonious tree for each gene region (Appendix C and E). *O. septentrionalis* from Hanmer was the most basal group, a second clade included *O. inaequalis* and a third encompassed populations of *O. aereus*. By combining gene regions the levels of bootstrap support were enhanced with a minimum of 79%. Maximum likelihood analysis (using the TVM+G model) produced a single tree (ln 2588.8792), (Figure 3.5), which was identical to the consensus parsimony tree (Figure 3.4). A three-way polytomy still existed between populations of *O. aereus* from the Dunedin, Rock and Pillar Range, Hokonui Hills and the Remarkables Range. The levels of bootstrap support were slightly lower for some clades compared with the parsimony tree, e.g., the node linking *O. inaequalis* and populations of *O. aereus*. However, these slightly lower bootstrap supports are insignificant given the same tree topology.

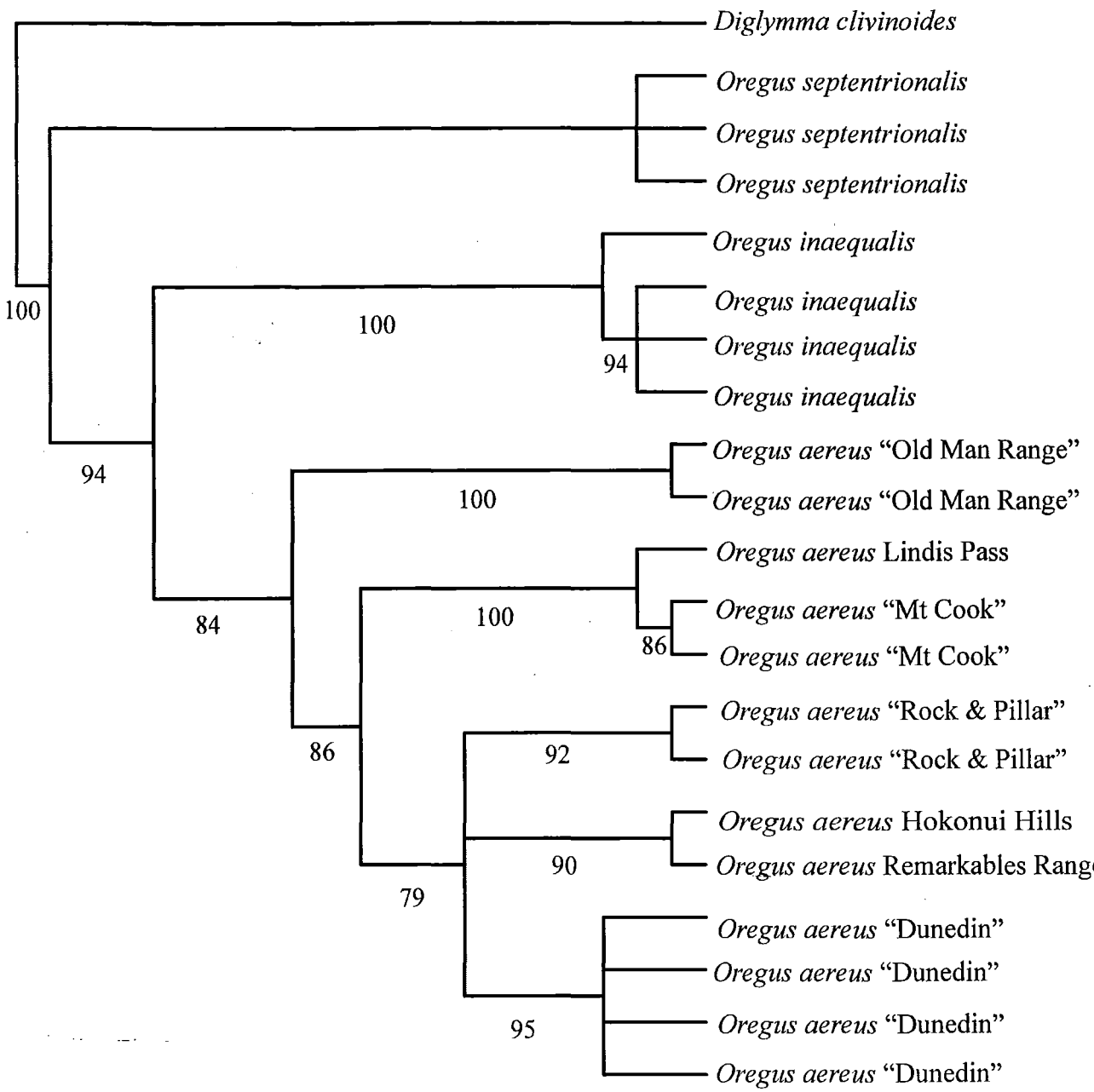


Figure 3.4. Strict consensus of two equally parsimonious phylogenetic trees (Length 210 steps, CI= 0.90, RI= 0.96) inferred from the combined COI and ND1 gene regions. Values below the branches indicate bootstrap supports from 1000 replicates.

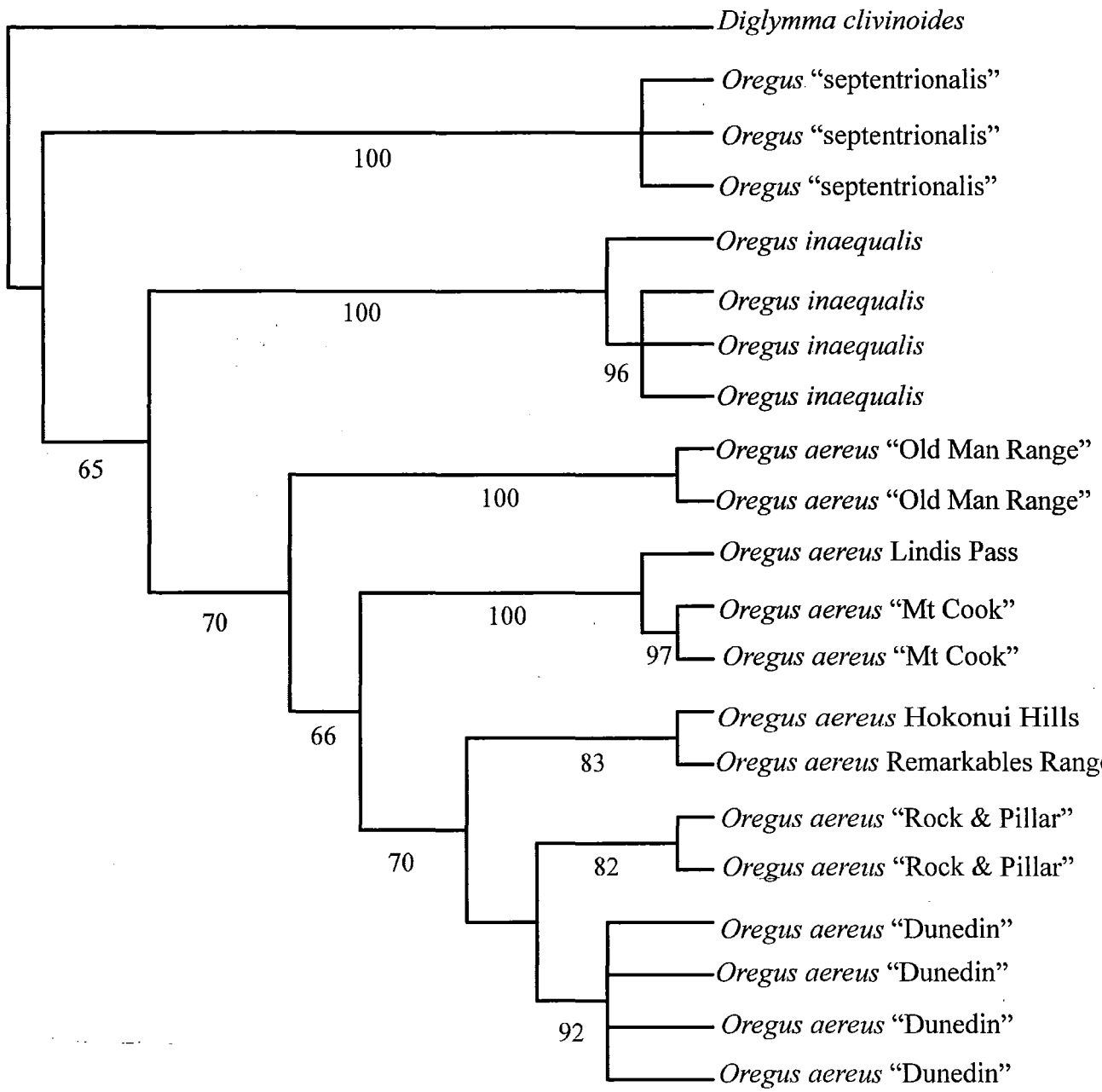


Figure 3.5. Maximum likelihood tree inferred from the combined COI and ND1 gene regions. Model of sequence evolution TVM + G, ln= 2588.8792, values below the branches indicate boot-strap support from 100 replicates.

Discussion

The analysis of the COI and ND1 gene regions supports at least three species in the genus *Oregus*. A fourth species (*O. crypticus*) described on the basis of morphology from museum specimens (Chapter 2) was not sequenced during the study, as no fresh specimens could be collected from the field. Based on morphology the results in Chapter 2 predict that *O. crypticus* would be most closely related to *O. septentrionalis*, the northern species and place *O. septentrionalis* as the sister taxon to *O. aereus*, with *O. inaequalis* most basal. Interestingly the molecular data place *O. septentrionalis* as the most basal species and *O. inaequalis* as the sister taxon to *O. aereus*. Corrected sequence divergence show *O. inaequalis* to be 3.2% distinct from *O. aereus,* which is a species that occurs in sympatry around Dunedin.

Analysis of the combined gene regions produced a monophyletic *O. aereus* clade with good bootstrap support (Figures 3.4 and 3.5). Specimens of *O. aereus* from the Old Man Range are the most basal and most genetically distinct population of this species (1.64-2.48 % COI and 1.15-1.87% ND1 different to other populations of *O. aereus*). The relationships between other populations of *O. aereus* are as expected given their geographical placement (Figure 3.2). The Lindis Pass population is geographically closest to the Mt Cook population and this is reflected in the phylogeny (Figure 3.4 and 3.5). The distinction between the Lindis Pass/Mt Cook populations and *O. aereus* from Dunedin, Rock and Pillars, Hokonui Hills and the Remarkables Range can be explained by their more northerly locality. Geographically intermediate populations would almost certainly be placed between these two nodes. However, additional faster evolving sequences would probably be necessary to resolve a geographical cline of such a fine scale. The placement of the Old Man Range population is interesting. Geographically it is closest to the Remarkable Range (Figure 3.2). However, both parsimony and maximum likelihood analyses indicate a relationship with the northern populations of *O. aereus* (Lindis Pass, Mt Cook, Figures 3.4 and 3.5), yet genetic distances indicate a close relationship to populations of *O. aereus* from the Pisa Range (COI) and the Hokonui Hills (ND1). The basal placement of the Old Man Range specimens amongst populations of *O. aereus* (and thus a close association with the Lindis Pass and Mt Cook populations of *O. aereus*, Figures 3.4 and 3.5) is probably influenced by long branch attraction to *O. inaequalis*, Figure 3.6, see discussion below.

The inconsistent placement of *O. inaequalis* in the COI maximum likelihood tree (Appendix B) contradicts my hypotheses based on morphology (chapter 2) and ND1 (that *O. inaequalis* is a distinct species) of species relationships, i.e., it suggests paraphyly in the *O. aereus* clade. Although maximum likelihood trees produced from the ND1 gene region and the combined (COI/ND1) analysis both place *O. inaequalis* as a sister taxon to a monophyletic clade of *O. aereus*, as expected. Another study using the COI gene region have shown conflicting results produced using maximum likelihood analysis with salticid spiders (Hedin and Maddison, 2001). However, in the present case it appears that long branch attraction has brought together *O. inaequalis* and *O. aereus* from the Old Man Range, based on COI data (Figure 3.6). Traditionally long branch attraction has been cited as an inconsistency problem associated with the parsimony method ((Hendy and Penny, 1989; Penny *et al.*, 1992), see Kim (1996) for an in-depth discussion) and that maximum likelihood, because of its ability to incorporate branch lengths, was not thought to be affected (Swofford *et al.*, 1996; Huelsenbeck, 1997). In this analysis it appears that maximum likelihood has been most affected by these unequal branch lengths (Appendix B) and this result is both unexpected and unexplained. The enhanced bootstrap support for the combined and ND1 gene regions, in combination with the basal placement of *O. inaequalis* by morphological data (Chapter 2), leads me to believe that the true placement of *O. inaequalis* is as a sister taxon to a monophyletic clade of *O. aereus*. Future attempts to overcome this case of long branch attraction should focus on the judicious inclusion of additional populations/species to breakup these long branches, see Kim (1996).

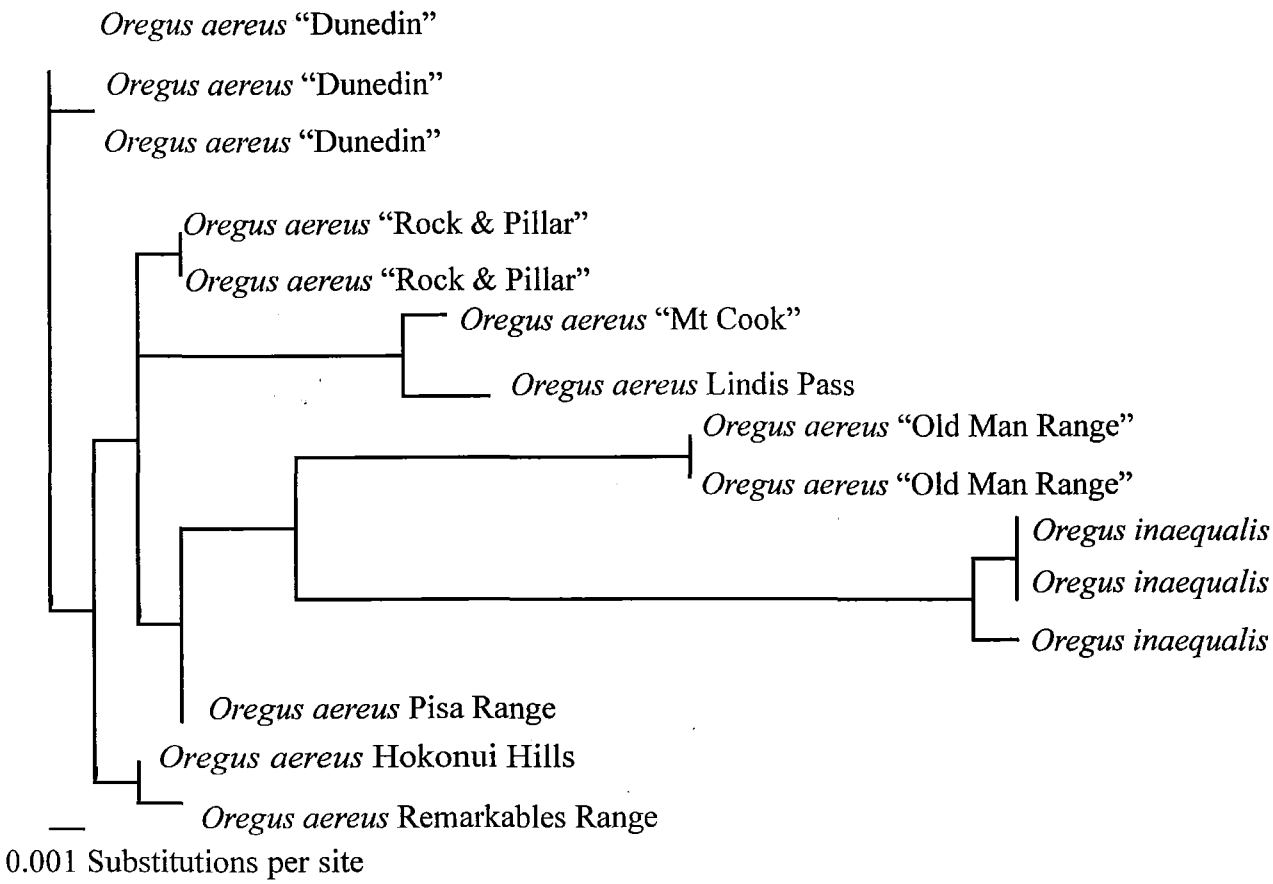


Figure 3.6. Maximum likelihood phylogram for *O. aereus* and *O. inaequalis* only of the COI gene region.

The COI and ND1 gene regions were chosen for their ability to resolve phylogenetic relationships at the species/population boundary (Simon, 1991; Pruser and Mossakowski, 1998; Duering and Bruckner, 2000). This choice was vindicated by corrected sequence divergence sufficient to distinguish between species and most populations. However, there was insufficient divergence to resolve the polytomy between populations of *O. aereus* from Dunedin, Rock and Pillar Range, Remarkables Range and Hokonui Hills. To achieve greater resolution a faster evolving gene region could be used. One possibility is to investigate the mitochondrial control region, although the rate of sequence evolution in the control region does vary between taxonomic groupings (Zhang and Hewitt, 1997). Increased confidence in the species relationships could be achieved by including a nuclear DNA marker. This approach was taken in similar work done by Sota and Vogler (2001) to examine incongruence between mitochondrial and morphological data in *Ohomopterus* carabids. The nuclear markers showed greater congruence with the morphological data in the case of *Ohomopterus* than the mitochondrial gene regions. However, in the current study

the morphological (Chapter 2) and molecular phylogenetic trees are largely congruent apart from the arrangement of the most basal taxa.

The placement of *Oregus septentrionalis* as the most basal species of *Oregus* leaves two options for the common ancestor of the genus. The most recent common ancestor could have had a northerly distribution and subsequently *O. aereus* and *O. inaequalis* colonised the southern region. Or alternatively, *O. septentrionalis* was isolated by vicariant means and evolved separately, north of a centre of origin located in the southern region of the South Island. However, further molecular evidence would need to be gathered from many populations to test these hypotheses.

Chapter 4. The Distribution of *Oregus inaequalis*, Its Abundance at Swampy Summit and Possible Threats to Its Future Survival.

Introduction

The conservation history of the New Zealand carabid fauna is one of a lack of information. Four species are presumed extinct and a further 47 species are considered threatened based on existing information about New Zealand's approximately 350 species of carabids (Molloy and Davis, 1994). Some of these are only known from a single series, often collected several decades ago, e.g., *Holcaspis brevicula* (Pawson and Emberson, 2000). Most threatened carabids in New Zealand belong to two large flightless genera, *Mecodema* and *Megadromus*. There have been few in-depth investigations to address the conservation requirements of New Zealand carabids and all involve the genus *Mecodema* or *Megadromus* (Barratt, 1993, 1994; Tennyson, 1998; Anderson, 2000).

Little is known about the distribution, abundance and population dynamics of New Zealand's apparently threatened carabid species (Moeed and Meads, 1985; Larochelle and Lariviere, 2001). However, the necessity for this information and the clarification of the taxonomy of these groups is recognised in the current draft carabid beetle recovery plan (McGuinness, 2001) and repeatedly in the literature, e.g., Lövei and Cartellieri (2000) and Ramsay *et al.* (1988). What is known about the conservation biology of these species, however, is the threat that they face from habitat reduction/modification (Lövei and Sunderland, 1996; Lövei and Cartellieri, 2000) and the influence of introduced mammalian predators. Large carabid beetles are particularly susceptible to predation from hedgehogs (Berry, 1999; Hendra, 1999), while rats have also been implicated in the decline of large invertebrate species (Hutcheson, 2000). However, few detailed studies, e.g., Lövei and Cartellieri (2000), have been conducted in New Zealand to quantify the effect of these threats on carabids, or investigate techniques to counteract them, e.g., Hunt *et al.* (1998).

Molloy and Davis (1994) placed *O. inaequalis* as a category B, or second priority species, in their prioritisation of New Zealand's threatened fauna. In response the Department of Conservation,

Otago Conservancy, commissioned a report (entitled "Existing records of the carabid beetle *Oregus inaequalis* Castlenau in coastal Otago" (Jamieson, 1999)) to collate existing historical records of *O. inaequalis* and provide recommendations for its conservation management. It is difficult to assess the historical distribution of *O. inaequalis* due to the vagaries of locality data from early specimens (Jamieson, 1999). However, *O. inaequalis* appeared to be restricted to Dunedin, Leith Valley, Swampy Summit, Mt Cargill, Waitati and Port Chalmers, all localities within Dunedin City. Several other specimens do not have any locality information.

Prior to this study nothing was known regarding the biology/phenology of larval or adult *O. inaequalis*, their present-day distribution, abundance or the effect on distribution of introduced mammalian predators. This chapter aims to extend the preliminary work of Jamieson (1999) and provide:

1. A re-inventory of the historical records of *O. inaequalis*, based on personal examination of all known and available specimens in New Zealand collections, and overseas collections where possible.
2. A comparison of historical records with the current distribution of *O. inaequalis* based on pitfall trapping and hand searching.
3. An estimate of the abundance of *O. inaequalis* at Swampy Summit.
4. A preliminary investigation of hedgehogs as potential predators of *O. inaequalis*.

Methods

Material was examined from collections, both institutional and private throughout New Zealand and overseas (Table 2.1). The list of known specimens of *O. inaequalis* was revised based on characters established by Britton (1949) and others developed as part of this study. This list (Appendix G) formed the basis for the historical distribution of *O. inaequalis*.

The current distribution of *O. inaequalis* was determined using unbaited pitfall traps (Appendix H) installed at various locations in the greater Dunedin area (four pitfalls per location) (Figure 4.2). Pitfall traps were left active for approximately one month (the time between sampling periods at Swampy Summit). Locations were chosen based on the historical distribution of *O. inaequalis*, and areas with similar habitat to Swampy Summit. Locations were recorded using a

Trimble GeoExplorer (Trimble Navigation, USA). Initially sodium benzoate was used as a preservative, however 10% ethylene glycol proved to be a less time-consuming and a more effective alternative and was used for the second and subsequent samples.

An attempt was made to estimate the abundance of *O. inaequalis* at Swampy Summit using a mark-removal method. A grid (8x10) of live pitfall traps (identical to those used to determine the current distribution of *O. inaequalis*) was installed on the 23rd August 2000. Pitfall traps were spaced at 20 m intervals, therefore the grid was 160m by 200m in size. The integrity of the grid was maintained by mapping it on the ground using tape measures and Pythagoras's theorem to obtain right angles. The four corners of the grid were located (using a Trimble GeoExplorer), in clockwise order from the south east, S 45° 47' 36.54" E 170° 28' 58.98", S 45° 47' 39.11" E 170° 28' 51.51", S 45° 47' 35.29" E 170° 28' 48.46" and S 45° 47' 32.62" E 170° 28' 55.80". The traps were left for one month prior to their first use to prevent possible bias in trap captures from soil disturbance during installation, a phenomena reported by Digweed *et.al.* (1995). The traps were activated for periods of five nights on five occasions (except for the last sampling period of four nights) from 23-27 September 2000, 29 October-2 November 2000, 11-15 December 2000, 8-12 January 2001 and 17-20 February 2001. *O. inaequalis* caught during the trapping period were identified using a hand lens to observe their antennal segments, supraorbital setae and fore tibial spines. Individuals were marked with a fine point silver marker (Pilot Super Colour Permanent Type Ink) and released in a random direction approximately 2 m from the trap. Anderson (2000) used a similar marking system with considerable success; as the markings seemed to be impervious to the fossorial habits of *Mecodema howiiti*, it was assumed that the markings would also be suitable for the closely related genus *Oregus*. Aggregation of captures was analysed using the Lloyds's Index of Patchiness (Davis, 1994). Because assumptions regarding normality and equal variance were not met, the nonparametric Kruskal-Wallis and Mann-Whitney U tests were used to assess the effects of soil moisture and proximity to boulders.

Minimum overnight temperatures were recorded using an electronic, max/min thermometer, with an accuracy of +/- 0.1 °C. Volumetric water content was recorded adjacent to each pitfall trap during each sampling period using a Hydrosense soil water meter.

Hedgehog faecal pellets were collected from Swampy Summit to determine possible predation of *O. inaequalis*. Faecal pellets were separated in warm water and detergent, they were then sieved

with a fine 250μM sieve to remove small particles. Coarser material was examined under a stereomicroscope using a Bogorov tray. Characteristic pieces such as tibia, elytral pieces, pronotum pieces, head capsules, antennal segments and mouthparts were separated and preserved in 70 % alcohol. These remains were then compared with mounted specimens to determine the presence of *Oregus*.

Elytral remains were collected from the burrows of ground spiders. These were compared with mounted specimens to determine whether ground spiders predated *Oregus*.

Results

Historic and Current Distribution

The historical distribution of *O. inaequalis* (Figure 4.1) is based on the personal examination of specimens known and available from national and international collections, a total of 78 individuals (Appendix G). A similar inventory by Jamieson (1999) included a number of specimens that, on re-examination during this study proved to be *O. aereus* rather than *O. inaequalis*. Britton (1949) listed *O. inaequalis* from Invercargill; examination of the specimen on which the record is based (held in NZAC) showed that it had been incorrectly identified and is in fact *O. aereus*. A specimen from the C.E. Clarke collection held in the Natural History Museum, London records locality information indicating that Mihiwaka and Port Chalmers were probably used synonymously. A similar situation indicates that specimens labelled Waitati are in fact from the slopes of Swampy Summit above Waitati (Jamieson, 1999). Anderson (2000) has also reported inconsistencies in C.E. Clarke's labelling of specimens of *Mecodema howiiti* and *Megadromus guerini* from Banks Peninsula.

Assuming that specimens labelled Port Chalmers really came from Mihiwaka, *O. inaequalis* was restricted in its historical distribution to flows from the third main eruptive phase of the Dunedin volcano (during the middle Miocene, 10-13 Ma) as mapped by Bishop and Turnball (1996). The distribution includes Swampy Summit, Leith Valley, and scattered locations across the volcanic plugs between Leith Saddle and Mihiwaka.

The current known distribution of *O. inaequalis* was determined using pitfall traps and hand searching. Little range contraction appears to have occurred in the last 100 years. *O. inaequalis* was found at all sites where previously recorded, except Mihiwaka (Figure 4.2). Although little range contraction was recorded during the study, there was a concurrent lack of extension to the known range. *O. inaequalis* was not recorded at any new sites, including Maungatua that has similar vegetation to Swampy Summit, but is noticeably drier. However, most pitfall trapping was concentrated at sites with historical records of *O. inaequalis*.

Figure 4.1. Historical records of *O. inaequalis* based on personal examination of known and available specimens.

Figure 4.2. Current distribution of *O. inaequalis* based on pitfall traps and hand collecting.

Estimate of *O. inaequalis* abundance at Swampy Summit

A total of 34 *O. inaequalis* were caught, marked and released in the pitfall trap grid on Swampy Summit during the five trapping periods (Figure 4.3). This amounts to a minimum population of 34 per 32, 000m^2. The distribution of trap captures throughout the grid is not random but significantly aggregated, 1.06 Lloyd's Index of Patchiness (Davis, 1994). The intention was to use a removal method of analysis such as Eberhardt's removal method (Krebs, 1999) to estimate population size. However, insufficient beetles were caught to obtain a realistic estimate of the population using this, or other removal methods. Beetles were recaught suggesting the population within the trapping grid is either much greater than 34 (Krebs, 1999), or alternatively captured individuals become trap shy very quickly, or perished as a result of handling.

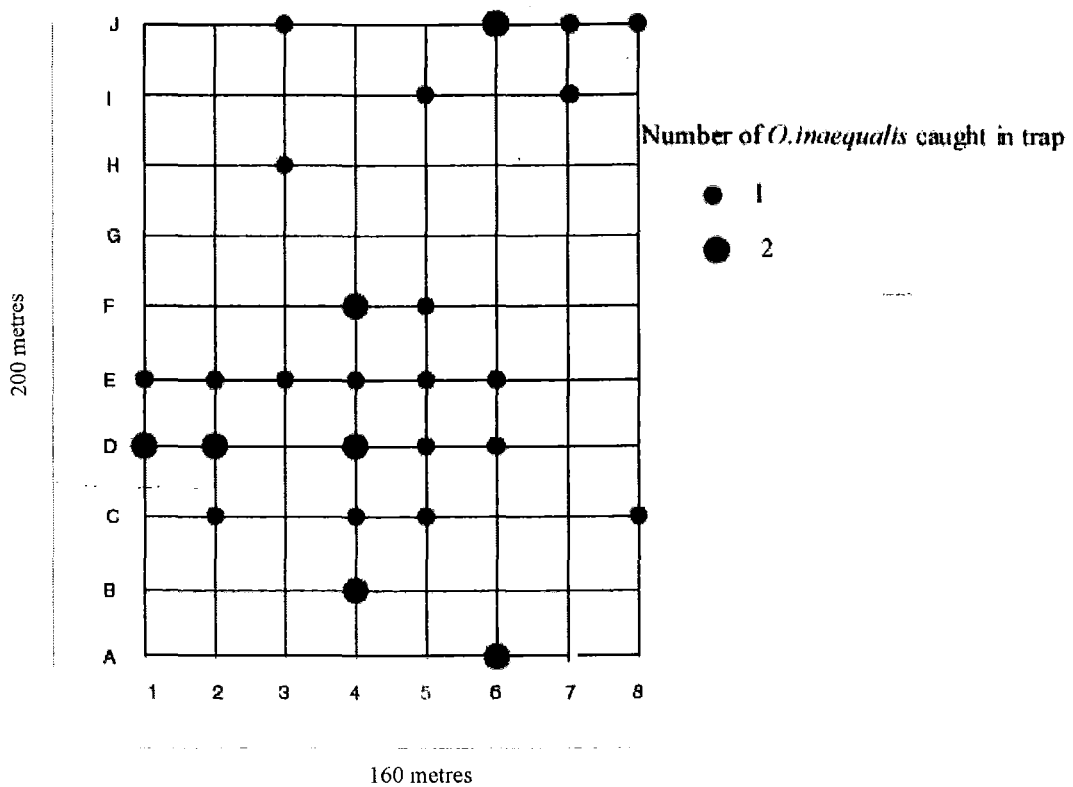


Figure 4.3. Distribution of pitfall trap captures of *O. inaequalis* at Swampy Summit during September-February.

Trap captures followed a distinctive seasonal pattern, with increasing carabid activity from late October through December, low activity in January that began increasing again in February (Figure 4.4). This activity closely followed the trend of minimum overnight temperatures. Other studies have shown similar responses in carabid activity that is significantly correlated to temperature, e.g., Honek (1997).

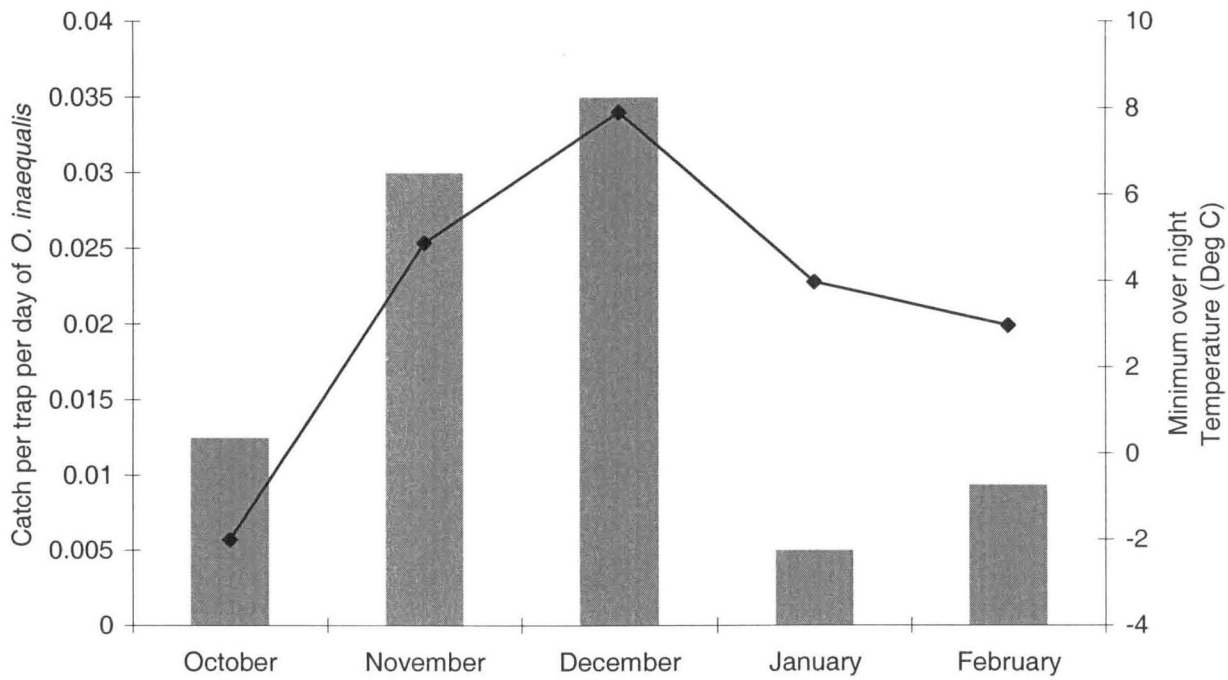


Figure 4.4. Monthly catch per trap per day of *O. inaequalis* (bars), and minimum monthly overnight temperatures (lines).

Trap capture was not significantly affected by soil moisture (P= 0.128, Kruskal-Wallis, df= 4). *O. inaequalis* was trapped at locations with soil moisture ranging from 55-96% (Figure 4.5). However, due to the vegetation types, e.g. sphagnum, individual beetles were probably not experiencing the high soil moisture (in some cases 90-100%) measured by the probe. Such differences in vegetation type may also have affected the catchability of *O. inaequalis*, as demonstrated by Greenslade (1964). Trap captures of *O. inaequalis* were almost twice as frequent when surface boulders were present in the immediate vicinity (< 3 m) of the trap (data not shown). This was

not statistically significant, P= 0.465 Mann-Whitney U, 1 df=1. due to the small number of beetles sampled and high variability.

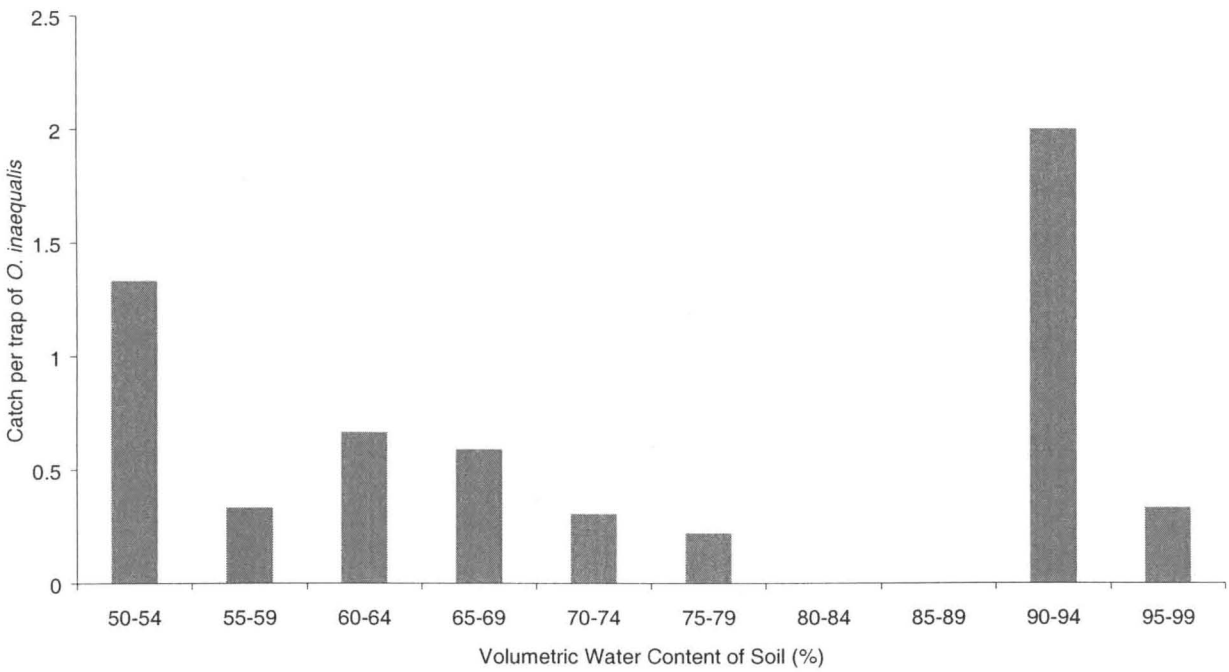


Figure 4.5. Influence of soil volumetric water content on rates of catch per trap of *O. inaequalis*.

Eight faecal samples from the European hedgehog were collected from Swampy Summit. Analysis of the remains did not conclusively identify *O. inaequalis* as a component of the diet. Remains of Coleoptera were common, including many Curculionidae, Scarabaeidae, Elateridae and Carabidae, including *Holcaspis* spp. and *Neoferonia* spp. which are of similar size to *Oregus*. Remains of both *O. inaequalis* and *O. aereus* were found in the nests of ground spiders, in some cases there were a dozen elytra in a single nest. This indicates that ground spiders may be an important predator of *Oregus*, as well as other Carabidae.

Discussion

The current distribution of *O. inaequalis* does not appear to have significantly contracted in comparison to historical records. *O. inaequalis* was not found at Mihiwaka in the present study, which was the most south-eastern historical record. A detailed re-survey of Mihiwaka is recom-

mended (using pitfall traps and hand surveying techniques) during November and December to confirm its presence/absence from the area.

The lack of extension to the current known range of *O. inaequalis* is not surprising as historical records of *O. inaequalis* are restricted to the mixed broadleaf podocarp forests that surrounds Dunedin. This forest type is only found elsewhere on Mount Pye in the Catlins. A single days searching on Mount Pye did not find any *O. inaequalis*. *O. inaequalis* during the study was found in the mixed broadleaf podocarp forested gullies that adjoin Leith Valley, e.g., Ross Creek, Morrison's Burn, as well as an unnamed tributary accessed via the Dunedin city water access bridge located at NZMS 260 I44 158 837 and in similar forest at Leith Saddle. This alters the historic perception that *O. inaequalis* is a tussock/shrubland adapted species. It is quite possible that *O. inaequalis* was originally a forest inhabitant and survives on Swampy Summit because of the W environment. The distribution of *O. inaequalis* may have been more extensive prior to the removal of large areas of broadleaf podocarp forest that were originally present in the Dunedin area. This possible larger historical distribution is unlikely to be recorded in the literature or museum collections, as locality information from the few specimens collected in the 1800's is very imprecise.

Swampy Summit, Mt Cargill and the sites in the Leith Valley are all on public land. Most is under the control of the Dunedin City Council and used for water catchment. As such it is afforded a degree of protection from urban development. Discouraging the removal of dead logs from the sites (particularly around Ross Creek Reservoir) would be beneficial to the continued survival of *O. inaequalis* at these sites.

Pitfall trap captures (as used in this study) are in themselves not a measure of abundance, but a measure of the biological activity of the species captured (Greenslade, 1964; Luff, 1975; Halsall and Wratten, 1988; Topping and Sunderland, 1992; Digweed *et al.*, 1995; Holland and Smith, 1999; Lang, 2000). A very common species may not be caught in pitfall traps simply because it is not active (and thus trappable) during the trapping period. As such it is not advisable to use pitfall trap captures alone as an estimate of abundance. The justification for this methodology is that a single species was involved; therefore variation in the biological activity and susceptibility to capture are irrelevant. Furthermore pitfall trap captures were not used as an absolute measure

of abundance, rather, they were used to provide samples for a catch removal method (Greenslade, 1964).

Pitfall trapping indicates that *O. inaequalis* is relatively common at Swampy Summit. Other pitfall trap studies of carabids, e.g., Butcher and Emberson (1981), show highly variable catch rates per species. At Ahuriri Bush, on Banks Peninsula, the most common carabid (*Mecodema oregoides*) was caught at a rate of 0.19-1.44 per trap per month, whereas the least common species (*Oopterus laevicollis*) was trapped at a rate of .0027 per trap per month (these figures have been converted to represent an equivalent number of pitfall traps to Swampy Summit). Trap captures of *O. inaequalis* ranged from 0.005-0.0125 per trap per month. Though not as common as some carabids of similar size, e.g., *M. oregoides*, or *Holcaspis* (which was the most abundant carabid at Swampy Summit and is slightly smaller in size), *O. inaequalis*, is more abundant on Swampy Summit than some other carabids are at other locations. However, this conclusion should be understood in the context that pitfall trap captures are strongly influenced by the activity of the species involved and the habitat within which the trapping is conducted. Unfortunately there is no data available on the relative surface activity of either *O. inaequalis* or *M. oregoides*, or the influence of habitat type on each species.

Further monitoring on a regular basis is not recommended at Swampy Summit or the adjoining broadleaf/podocarp forest gullies. However, it would be prudent to monitor following any major disturbance, e.g., fire or deforestation, to determine the resilience of known populations. Development within the known distribution of *O. inaequalis*, especially the forested remnants of the Leith Valley, should be discouraged to prevent fragmentation of important forest refugia, a process known to affect carabid assemblages (Lövei and Cartellieri, 2000). This is especially important as this forest type may be the original habitat for *O. inaequalis*. When necessary sampling/monitoring of *O. inaequalis* should be done during the months of November and December as this corresponds to the known period of greatest surface activity.

O. inaequalis appeared to be aggregated in its distribution and possibly influenced by the presence of surface boulders. The use of boulders and logs as daytime refugia is well known in other carabids (Griffiths, 1983; Barratt, 1993, 1994; Larochelle and Lariviere, 2001). However, Swampy Summit is an incredibly diverse shrubland/tussock community with a complex, heterogeneous surface environment. Such an environment provides many other sources of daytime

refugia, e.g., tussock mounds (Luff, 1966). Habitat modification is recognised as a factor in the decline of carabid beetles (Lovei and Cartellieri, 2000). Gorse (*Ulex europaeus*) and broom (*Cytisus scoparius*) are widely, but sparsely, distributed across Swampy Summit. The effect of these invasive weeds on *O. inaequalis* is unknown, but they will undoubtedly have a significant effect on the microclimate and microhabitat, including prey availability. It is therefore important to prevent the invasion and subsequent dominance of the habitat by gorse and broom.

The activity period of *O. inaequalis*, as shown by pitfall trapping, is typical of many carabid species. A period of high spring and early summer activity, low levels of activity during mid-summer and a subsequent increase of autumn activity is typical of many carabids, both overseas (Thiele, 1977) and in New Zealand (Anderson, 2000). The relationship between trap capture of *O. inaequalis* and moisture is difficult to interpret. It seems very unlikely that these beetles are surviving in areas with 90-100% soil moisture. It is likely that the complex surface architecture of vegetation allows *O. inaequalis* to move freely between areas of high and low soil moisture.

Coleoptera remains were present in the faecal samples of hedgehogs, including two carabid species (*Holcaspis* spp., *Neoferonia* spp.) of similar size to *Oregus*, and large numbers of Curculionidae, some Scarabaeidae and Elateridae. No recognisable remains of either *O. inaequalis* or *O. aereus* were observed. Unfortunately this does not preclude hedgehogs as a significant predator of *Oregus*. *Holcaspis* is more abundant than *Oregus* and more likely to be present in the limited faecal samples examined. These results do imply that hedgehogs are not exclusively preying on *Oregus* or favouring them over *Holcaspis*. Given that they are known to feed on other carabids at Swampy Summit it is highly likely hedgehogs predate upon *O. inaequalis*. Substantial further sampling would be required before hedgehogs could, with confidence, be eliminated as a predator of *O. inaequalis*. Future attempts at determining the predation of *Oregus* by hedgehogs may like to consider the application of DNA techniques to identify species-specific remains in gut contents or faeces (Farrell *et al.*, 2000). Zaidi *et al.* (1999) has developed techniques to determine the stomach contents of carabids and there is potential to extend this to hedgehogs.

Ground spiders are definitely predators of *O. inaequalis* and other carabids, e.g., *Holcaspis*, *Mecodema* and *Neoferonia*. Ground spiders and Carabidae have coexisted for a substantial period of time in New Zealand and it is unlikely that predation by spiders has increased during post human colonisation of New Zealand. However, the effect of habitat change on the abundance of ground

spiders is unknown and cannot be quantified. As such spiders are not thought to represent a serious potential threat to the survival of *O. inaequalis*.

Chapter 5 General Discussion

This thesis investigated species relationships within the genus *Oregus* Putzeys 1868 as well as aspects of the distribution and abundance of one species, *O. inaequalis* Castlenau 1867. There is a current national and international focus on the recognition of, and subsequent preservation of biodiversity (Department of Conservation and Ministry for the Environment, 2000). Such basic taxonomic, systematic and biological research was necessary to provide the Department of Conservation with information to facilitate quality conservation management of *Oregus*.

Both morphological and molecular techniques were used to examine species relationships within *Oregus*. Morphology was important, as it was necessary to recognize characters suitable for accurate field identification of *O. inaequalis*. Due to the morphological conservatism of carabids as a group, molecular techniques were included to enhance confidence in the inferred phylogeny (de Queiroz *et al.*, 1995).

Since 1873, the genus *Oregus* has contained two species (Britton, 1949; Putzeys, 1873), however, in recent years there has been some controversy over the exact number of species. Barbara Barratt (personal communication, 2001) had to convince others that *O. inaequalis* was not a synonym of *O. aereus*, and Jamieson (1999) suggested the possibility of additional species around the Dunedin Metropolitan area. Obviously a detailed comprehensive morphological study was required. The conservative external body morphology led to a reliance on male genitalic characters, which were less homoplasious than either female genitalic characters or external morphology (Kruskal-Wallis test, $P<0.001$). Morphological analysis indicated four divisions within the genus, two new species from the upper South Island (manuscript names *Oregus septentrionalis* and *Oregus crypticus*), *O. inaequalis* from the Dunedin peri-urban area and a widely distributed intra-specifically variable *O. aereus*, collected from Porters Pass, MC to Invercargill, SL. The new, northern species were identified on the basis of characters associated with the shape of the tip of the aedeagus, characteristics of the left and right paramere and the presence of setiferous punctures either side of the midline of ventrite 6. Unfortunately there are no defining external characters to separate these two northern species. *O. inaequalis* is easily identified by its moniliform antennae, increased numbers of supraorbital setae and characteristics of the sclerotised projections of the apical plate in the male genitalia.

The molecular approach to the phylogeny of *Oregus* utilised sequences from mitochondrial gene regions to capitalise on the larger data sets that contain many variable characters suitable for the analysis of populations and closely related species (Simon *et al.*, 1994; Swofford *et al.*, 1996). The more variable COOH terminal region of COI gene region (Lunt *et al.*, 1996) and the rapidly evolving ND1 gene region were sequenced. The COI gene region was PCR amplified with little difficulty using primers C1-J-2195 and TL2-N-3014, which produced good sequencing products. The ND1 gene region was more difficult to PCR amplify. Initial use of primer N1-J-12314 (Pashley and Ke, 1992; Pruser and Mossakowski, 1998) was inconsistent and often produced faint bands (attributed to sequence variation at the N1-J-12314 priming site). However subsequent use of N1-J-12261 (Hedin, 1997) in combination with LR-N-12866 consistently produced strong bands and good sequencing products (except for a nontarget product amplified from the Pisa Range population of *O. aereus*). Such problems with non-target amplification could be resolved by designing new PCR primers from the sequence data generated in the study.

The molecular work was restricted, as fresh material of *O. crypticus* was not collected during the study. However, the material sequenced indicated three divisions within the genus, which supported the morphological species designations. *O. aereus* had the greatest intraspecific sequence variation, probably reflecting its much wider distribution in comparison to the other species. However, the intraspecific variation observed was much less than interspecific variation lending further support for the divisions between species. There was some conflict between the morphological and molecular analysis as to whether *O. inaequalis* or *O. septentrionalis* was the basal taxon in the genus. This could not be resolved. However previous studies have shown incongruence between mitochondrial and morphological analyses of carabid beetles (Su *et al.*, 1996b). Following the study of Su *et al.* (1996b) the use of nuclear gene markers by Sota and Vogler (2001) showed better congruence with morphological analyses. Similarly I would recommend attempting to use nuclear molecular markers if it was seen necessary to resolve this basal node.

The results of this thesis make an important contribution to the taxonomy of *Oregus*. It confirms *O. inaequalis* as a distinct taxonomic entity. Therefore it can be considered as a conservation management unit by the Department of Conservation. Jamieson's (1999) suggestion of new species in the Dunedin area proved to be unfounded. However, the study did highlight the existence of new species in the upper South Island and the presence of considerable genetic diversity be-

tween populations of *O. aereus*. This begs the question, what of the taxonomy of other broscine carabids, particularly *Mecodema*? The taxonomy of the New Zealand Broscini as a whole is relatively poor with no comprehensive revision since Britton in 1949. A detailed taxonomic study such as this one would undoubtedly discover many new species within this tribe and identify new synonyms.

The results of DNA sequencing highlight several useful additions that molecular techniques can make to the areas of taxonomy and phylogeny. Without DNA sequencing the high genetic divergence (and thus the degree of anagenesis) of *O. septentrionalis* would not have been detected. Many studies across a range of taxonomic groups have shown the usefulness of molecular techniques in identifying such hidden genetic diversity (Jousson *et al.*, 2000; Shaw, 2000; Trewick, 2000). The identification of genetic diversity is important especially if a phylogenetic approach is taken to conservation management (Soltis and Gitzendanner, 1999).

The historical published distribution of *O. inaequalis*, included specimens from Invercargill, S.L (Britton, 1949) and unconfirmed records from Lake Pukaki, M.K and Fox's Peak, S.C (Jamieson, 1999). Personal examination of all available specimens from New Zealand collections and those known from international collections highlighted many misidentifications. Despite the vagaries of many early locality labels the historic distribution of *O. inaequalis* appears to have been restricted to the hills immediately to the North of Dunedin city. Records from Southland were misidentifications and no specimens seen from either the Mackenzie Basin or South Canterbury proved to be *O. inaequalis*. Pitfall trapping during this study, which proved a very effective method of collecting *O. inaequalis* recollected specimens from all known historical localities except Mihiwaka. The current distribution of *O. inaequalis* is restricted to the subalpine tussock, shrubland and broadleaf/kaikawaka forests that dominate the hills around Swampy Summit, Leith Saddle, Mt Cargill and the forested valleys that extend from them. Ross Creek Reservoir was the most urbanised locality of the current distribution.

An 8x10 grid of pitfall traps was installed on Swampy Summit to collect *O. inaequalis* in an attempt to estimate abundance using a mark-removal or a mark-recapture method. Only 34 individuals were collected and no marked individuals were recaptured. This was insufficient for any quantitative estimate of abundance to be made. It was soon apparent that the only efficient method of collecting *O. inaequalis* in the habitat was pitfall trapping therefore any accurate future

quantitative estimates of abundance are unlikely. Trap captures did show seasonal variation with most beetle activity in spring and early summer, such patterns of activity are common amongst carabids (Anderson, 2000; Thiele, 1977). Trap captures appeared to be aggregated around groups of boulders, however this was statistically not significant. Based on the very complex micro-topography and vegetation surrounding the pitfall traps, I would estimate the probability of catching an individual is low. Based on this low probability of capture, the number I did catch (34) and the fact that *O. inaequalis* was the second most commonly caught carabid after *Holcaspis* (which may have been multiple species as *H. impiger* and *H. placida* are only distinguishable on the basis of male genitalia (Butcher, 1984)) I believe *O. inaequalis* is relatively abundant at Swampy Summit.

Because *O. inaequalis* has not shown any significant range contraction, appears to be relatively abundant (at least at Swampy Summit) and that most of its habitat is protected for Dunedin City water catchment I do not recommend any conservation management for the species. However the newly designated *Oregus crypticus* is known only from a few specimens; the most recent collected is from the Eryewell forest in 2001. Most localities where it has been collected are significantly modified for pastoralism or forestry. *O. crypticus* is possibly adapted to tussock shrubland ecosystems, large areas of which have declined in both area and quality throughout North Canterbury. A current survey is urgently required to determine the present distribution of *O. crypticus*. It is important to assess whether any of its habitat is currently protected, as most land in the region is used for pastoralism.

Although this study failed to provide a quantitative estimate of the abundance of *O. inaequalis* at Swampy Summit, it did quite importantly clarify the distribution of this species. The historical distribution of *O. inaequalis* has been debated in the literature (Jamieson, 1999) and in unpublished discussions between entomologists. This study confirms that *O. inaequalis* is a narrow range endemic, restricted to the hills immediately to the North of Dunedin City. As such *O. inaequalis* becomes somewhat of an iconic species for Dunedin.

Thesis Outcomes

i. This thesis aimed to identify the species relationships within the genus *Oregus*. Morphological and molecular analysis's indicates four species, two of which are described for the

first time in this thesis. Morphological characters were defined that allow accurate and unequivocal field identification of *O. inaequalis* for the first time. This information was conveyed to the Department of Conservation in a report entitled Pawson, S. M. and Emberson, R.M. (2001). *Oregus inaequalis* Castelnau, its distribution, and abundance at Swampy Summit. *DOC Science Internal Series* **6**, 1-20.

ii. The study showed no significant historical range contraction of *O. inaequalis*. The current distribution of *O. inaequalis* is associated with subalpine tussock, scrubland and broadleaf forest communities. The restricted range appears to be natural and not human induced. This work failed to make any quantitative estimate of abundance, however all evidence suggests that *O. inaequalis* is abundant, at least at Swampy Summit. *O. inaequalis* whilst unusually geographically restricted is not considered to be severely threatened based on these results.

Chapter 6 References

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., *et al.* (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research 25*:3389-33402.

Anderson, S. J. (2000). *Distribution, habitat associations, and activity patterns of two endemic Banks Peninsula carabid beetles,* Mecodema howiiti *and* Megadromus australasiae. Unpublished Master of Applied Science, Lincoln University, Lincoln.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., *et al.* (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics 18*:489-522.

Ball, G. (1979). Conspectus of carabid classification: History, holomorphology, and higher taxa. Pages 63-112 In (T. L. Erwin, Ball, G. E., Whitehead, D. R., and Halpern, A. L., eds.). *Carabid beetles: their evolution, natural history, and classification* Dr W.Junk, Hague.

Ball, G. E. (1956). Notes on the genus *Zacotus* Le Conte, 1869, and on the classification of the tribe Broscini (Broscidae sensu Jeannel, 1941. Coleoptera, Carabidae). *The Coleopterists' Bulletin 10*:33-52.

Barratt, B. I. P. (1993). *Mecodema chiltoni* Broun an assessment of the priority for conservation. *Conservation Advisory Science Notes 14*:21.

Barratt, B. I. P. (1994). *Mecodema laeviceps* Broun an assessment of the priority for conservation. *Conservation Advisory Science Notes 74*:13.

Barratt, B. I. P., and Patrick, B. H. (1987). Insects of the snow tussock grasslands on the East Otago Plateau. *New Zealand Entomologist 10*:69-98.

Bensasson, D., Zhang, D.-X., Hartl, D. L., and Hewitt, G. M. (2001). Mitochondrial pseudogenes: evolutions misplaced witnesses. *Trends in Ecology and Evolution 16*:314-321.

Berry, C. (1999). Potential interactions of hedgehogs with Northern Island Brown Kiwi at Boundry Stream Mainland Island. *Conservation Advisory Science Notes 268.*

Bishop, D. G., and Turnbull, I. M. (1996). Geology of the Dunedin area. Institute of Geological and Nuclear Sciences 1:250 000 geological map 21New Zealand Institute of Geological and Nuclear Sciences Limited, Lower Hutt.

Britton, E. B. (1949). The Carabidae (Coleoptera) of New Zealand Part III A revision of the tribe Broscini. *Transactions of the Royal Society of New Zealand 77*:533-581.

Brower, A. V. Z., and DeSalle, R. (1994). Practical and theoretical considerations of choice of a DNA sequence region in insect molecular systematics, with a short review of published studies using nuclear gene regions. *Annals of the Entomological Society of America 87*:702-716.

Brown, B. (1998). *The evolutionary history of the 'Oxycanus' lineages of hepialid moths in New Zealand* Unpublished PhD, Lincoln University, Lincoln

Brown, B. (2000). Morphological character evolution in hepialid moths (Lepidoptera: Hepialidae) from New Zealand. *Biological Journal of the Linnean Society 69*:383-397.

Brown, J. M., Pellmyr, O., Thompson, J. N., and Harrison, R. G. (1994). Phylogeny of *Greya* (Lepidoptera: Perodoxidae), based on nucleotide sequence variation in mitochondrial cytochrome oxidase I and II: congruence with morphological data. *Molecular Biology and Evolution 11*:128-141.

Bull, J. J., Huelsenbeck, J. P., Cunningham, C. W., Swofford, D. L., and Waddell, P. J. (1993). Partitioning and combining data and phylogenetic analysis. *Systematic Biology 42*:384-397.

Butcher, M. R. (1984). A revision of the genus *Holocaspis*(Coleoptera: Carabidae). *Journal of the Royal Society of New Zealand 77*:533-581.

Butcher, M. R., and Emberson, R. M. (1981). Aspects of the biology of carabid beetles of Ahuriri Bush Scenic Reserve Banks Peninsula. *Mauri Ora 9*:59-70.

Castelnau, C. L. (1867). Notes on Australian Coleoptera. *Separates available prior to publication in 1868*. See next reference.

Castelnau, C. L. (1868). Notes on Australian Coleoptera. *Transactions of the Royal Society of Victoria 8*:95-225.

Caterino, M. S., Cho, S., and Sperling, F. A. H. (2000). The current state of insect molecular systematics: A thriving tower of Babel. *Annual Review of Entomology*:1-55.

Chenu, J. C. (1851). *Encyclopedie d'histoire naturelle ou Traite complet de cette science d'apres les travaux des naturalistes les plus eminents de tous les pays et de toutes les epoques.Coleopteres, avec la collaboration de M.E. Desmarest.* Marescq and Harvard, Paris.

Clary, D. O., and Wolstenholme, D. R. (1985). The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organisation, and genetic code. *Journal of Molecular Evolution 22*:252-271.

Conservation, Department of., and Environment, Ministry of. (2000). *The New Zealand Biodiversity Strategy: Our chance to turn the tide. Whakakohukihukitia Te Tai Roroku Ki Te Tai Oranga*, Wellington.

Crosby, T. K., Dugdale, J. S., and Watt, J. C. (1998). Area codes for recording specimen localities in the New Zealand subregion. *New Zealand Journal of Zoology 25*:175-83.

Davis, P. M. (1994). Statistics for describing populations. Pages 33-54 In (L. P. Pedigo, Butin, G.D., ed.) *Handbook of sampling methods for arthropods in agriculture* CRC Press.

de Queiroz, A. D. (1993). For consensus (sometimes). *Systematic biology 42*:368-372.

de Queiroz, A. D., Donoghue, M. J., and Kim, J. O. (1995). Separate versus combined analysis of phylogenetic evidence. *Annual Review of Ecology and Systematics 26*:657-681.

Dickinson, K. J. M. (1988). Umbrella Ecological District: a survey report for the New Zealand protected areas program. Pages 179 Department of Conservation, Wellington.

Digweed, S. C., Currie, C. R., Carcamo, H. A., and Spence, J. R. (1995). Digging out the "digging-in effect" of pitfall traps: influences of depletion and disturbance on catches of ground beetles (Coleoptera: Carabidae). *Pedobiologia 39*:561-576.

Duering, A., and Bruckner, M. (2000). The evolutionary history of the tribe Molopini: a first molecular approach. Pages 1-4 In (P. Brandmayer, ed.) *Natural history and applied ecology of carabid beetles* Pensoft, Sofia.

Dupuis, C. (1984). Willi Hennig's impact on taxonomic thought. *Annual Review of Ecology and Systematics 15*:1-24.

Edwards, A. W. F., and Cavalli-Sforza, L. L. (1964). Reconstruction of evolutionary trees. Pages 67-76 In (V. H. Heywood, and McBeil, J., eds.). *Phenetic and phylogenetic classification* Systematics Association, London.

Erwin, T. L. (1991). The ground-beetles of Central America (Carabidae), part II: Notiophilini, Loricerini and Carabini. *Smithsonian Contributions to Zoology 501*:30.

Farrell, L. E., Roman, J., and Sunquist, M. E. (2000). Dietary separation of sympatric carnivores identified by molecular analysis of scats. *Molecular Ecology 9*:1583-.

Farris, J. S. (1989). The retention index and the rescaled consistency index. *Cladistics 5*:417-419.

Farris, J. S., Kallersjo, M., Kluge, A. G., and Bult, C. (1995). Testing significance of incongruence. *Cladistics 10*:315-319.

Felsenstein, J. (1978). Cases in which parsimony or compatibility methods will be positively misleading. *Systematic Zoology 27*:401-410.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution 17*:368-376.

Fitch, W. M. (1971a). Towards defining the course of evolution: minimum change for a specified tree topology. *Systematic Zoology 19*:172-189.

Fitch, W. M. (1971b). Towards defining the course of evolution: minimum changes for a specified tree topology. *Systematic Zoology 20*:406-16.

Funk, D. J. (1999). Molecular systematics of cytochrome oxidase I and 16S from *Neochlamisus* leaf beetles and the importance of sampling. *Molecular Biology and Evolution 16*:67-82.

Futuyma, D. J. (1998). *Evolutionary Biology*, 3rd edition. Sinauer Associates Inc, Sunderland.

Galian, J., De la Rua, P., Serrano, J., Juan, C., and Hewitt, G. M. (1999). Phylogenetic relationships in West Mediterranean Scaritina (Coleoptera: Carabidae) inferred from mitochondrial COI sequences and karyotype analysis. *Journal of Zoological Systematics and Evolutionary Research 37*:85-92.

Gemminger, M., and Harold, E. v. (1868). *Catologus coleopterorum hucusque descriptorum synonymicus et systematicus.* Gummi, Monachii.

Goldman, N. (1993a). Simple diagnostic statistical tests for models for DNA substitution. *Journal of Molecular Evolution 37*:650-661.

Goldman, N. (1993b). Statistical tests of models of DNA substitution. *Journal of Molecular Evolution 36*:182-198.

Gray, M. W., Burger, G., and Lang, B. F. (1999). Mitochondrial evolution. *Science 283*:1476-1481.

Greenslade, P. J. M. (1964). Pitfall trapping as a method of studying populations of Carabidae (Coleoptera). *Journal of Animal Ecology 33*:301-310.

Griffiths, E. (1983). *The feeding ecology of the carabids beetle* Agonum *dorsale in cereal crops* Unpublished PhD, University of Southampton, Southampton

Gyllensten, U., Wharton, D., Josefsson, A., and Wilson, A. C. (1991). Paternal inheritance of mitochondrial DNA in mice. *Nature 352*:255-257.

Halsall, N. B., and Wratten, S. D. (1988). The efficiency of pitfall trapping for polyphagous predatory Carabidae. *Ecological Entomology 13*:293-299.

Hasegawa, M., Kishino, H., and Yano, T. (1985). Dating the human-ape split by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution 22*:160-174.

Hedin, M. C. (1997). Molecular phylogenetics at the population/species interface in cave spiders of the Southern Appalachians (Araneae:Nesticidae: *Nesticus*). *Molecular Biology and Evolution 14*:309-324.

Hedin, M. C., and Maddison, W. P. (2001). A combined molecular approach to phylogeny of the jumping spider subfamily Dedryphantinae (Araneae: Salticidae). *Molecular Phylogenetics and Evolution 18*:383-403.

Hendra, R. (1999). Seasonal abundance patterns and dietary preferences of hedgehogs at Trounson Kauri Park. *Conservation Advisory Science Notes 267.*

Hendy, M. D., and Penny, D. (1989). A framework for the quantitative study of evolutionary trees. *Systematic Zoology 38*:297-309.

Hennig, W. (1966). *Phylogenetic Systematics.* University of Illinois Press, Chicago.

Hewitt, G. M. (2001). Speciation, hybrid zones and phylogeography or seeing genes in space and time. *Molecular Ecology 10*:537-550.

Hillis, D. M. (1987). Molecular versus morphological approaches to systematics. *Annual Review of Ecology and Systematics 18*:23-42.

Hillis, D. M. (1991). Discriminating between phylogenetic signal and random noise in DNA sequences. Pages 278-294 In (M. M. Miyamoto, and Cracraft, J., eds.). *Phylogenetic analysis of DNA sequences* Oxford University Press, Oxford.

Hillis, D. M. (1995). Approaches for assessing phylogenetic accuracy. *Systematic Biology 44*:3-16.

Hillis, D. M., and Huelsenbeck, J. P. (1992). Signal, noise and reliability in molecular phylogenetic analyses. *The Journal of Heredity 83*:189-195.

Holland, J. M., and Smith, S. (1999). Sampling epigeal arthropods: an evaluation of fenced pitfall traps using mark-release-recapture and comparisons to unfenced pitfall traps in arable crops. *Entomolgia Experimentalis et Applicata 91*:347-357.

Honek, A. (1997). The effect of temperature on the activity of Carabidae (Coleoptera) in a fallow field. *European Journal of Entomology 94*:97-104.

Howland, D. E., and Hewitt, G. M. (1995). Phylogeny of the Coleoptera based on mitochondrial cytochrome oxidase I sequence data. *Insect Molecular Biology 4*:203-215.

Huelsenbeck, J. P. (1991). Tree-length distribution skewness: An indicator of phylogenetic information. *Systematic Zoology 40*:257-270.

Huelsenbeck, J. P. (1997). Is the Felsenstein zone a fly tap? *Systematic Biology 46*:69-74.

Huelsenbeck, J. P., Bull, J. J., and Cunningham, C. W. (1996). Combining data in phylogenetic analysis. *Trends in Ecology and Evolution 11*:152-158.

Huelsenbeck, J. P., and Crandall, K. A. (1997). Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics 28*:437-466.

Hull, D. L. (1965). Certainty and circularity in evolutionary taxonomy. *Evolution 21*:174-189.

Hull, D. L. (1988). *Science as a process: An evolutionary account of the social and conceptual development of science*. The University of Chicago Press, Chicago.

Hunt, M., Sherley, G., and Wakelin, M. (1998). Results of a pilot study to detect benefits to large-bodied invertebrate from sustained regular poisoning of rodents and possums at Karioi, Ohakune. *Science for Conservation 102*:18.

Hutcheson, J. A. (2000). Invertebrate fauna and their ecological context on Whangaokena, East Cape. *Conservation Advisory Science Notes 324*:43.

Jamieson, C. (1999). Existing records of the carabid beetle *Oregus inaequalis* Castelnau in coastal Otago. *Conservation Advisory Science Notes 244*:12.

Jeannel, R. (1941). Coleopteres Carabiques Part 1. *Faune de France 39*:1-571.

Jones, T. R., Kluge, A. G., and Wolf, A. J. (1993). When theories and methodologies clash: A phylogenetic reanalysis of the North American Ambystomatid Salamanders (Caudata: Ambystomatidae). *Systematic Biology 42*:92-102.

Jousson, Bartoli, and Pawlowski. (2000). Cryptic speciation among intestinal parasites (Trematoda: Diagenea) infecting sympatric host fishes (Sparidae). *Journal of Evolutionary Biology 13*:778.

Juan, C., Ibrahim, K. M., Oromi, P., and Hewitt, G. M. (1996a). Mitochondrial DNA sequence variation and phylogeography of *Pimelia* darkling beetles on the island of Tenerife (Canary Islands). *Heredity 77*:589-598.

Juan, C., Oromi, P., and Hewitt, G. M. (1996b). Phylogeny of the genus *Hegeter* (Tenebrionidae, Coleoptera) and its colonisation of the Canary Islands deduced from cytochrome oxidase I mitochondrial DNA sequences. *Heredity 76*:392-403.

Jukes, T. H., and Cantor, C. R. (1969). Evolution of protein molecules. Pages 21-132 In (H. N. Munro, ed.)*Mammalian Protein Metabolism* Academic Press, New York.

Kambhampati, S., and Smith, P. T. (1995). PCR primers for the amplification of four insect mitochondrial gene fragments. *Insect Molecular Biology 4*:233-236.

Kelly, S. T., Mitton, J. B., and Paine, T. D. (1999). Strong differentiation in mitochondrial DNA of *Dendroctonus brevicomis* (Coleoptera: Scolytidae) on different subspecies of Ponderosa pine. *Annals of the Entomological Society of America 92*:193-197.

Kim, J. (1996). General inconsistency conditions for maximum parsimony: Effects of branch lengths and increasing numbers of taxa. *Systematic Biology 45*:363-374.

Kitching, I. J., Forey, P. L., Humphries, C. J., and Williams, D. M. (1998). *Cladistics: the theory and practice of parsimony analysis*. Oxford University Press, Oxford.

Kluge, A. G., and Farris, J. S. (1969). Quantitative phyletics and the evolution of Anurans. *Systematic Zoology 18*:1-32.

Kluge, A. G., and Wolf, A. J. (1993). Cladistics: What's in a word? *Cladistics 9*:183-199.

Kondo, R., Satta, Y., Matsuura, E. T., Ishiwa, H., Takahata, N., and Chigusa, S. I. (1990). Incomplete maternal transmission of mitochondrial DNA in *Drosophila*. *Genetics 126*:657-663.

Krebs, C. J. (1999). *Ecological methodology*, 2nd edition. Addison-Welsey Educational Publishers.

Lang, A. (2000). The pitfalls of pitfalls: a comparison of pitfall trap catches and absolute density estimates of epigeal invertebrate predators in arable land. *Journal of Pest Science 73*:99-106.

Langor, D. W., and Sperling, F. A. H. (1997). Mitochondrial DNA sequence divergence in weevils of the *Pissodes strobi* species complex (Coleoptera: Curculionidae). *Insect Molecular Biology 6*:255-265.

Larochelle, A., and Lariviere, M.-C. (2001). Carabidae (Insecta: Coleoptera): catalogue. *Fauna of New Zealand 43*:285.

Lewis, P. O. (2001). Phylogenetic systematics turns over a new leaf. *Trends in Ecology and Evolution 16*:30-37.

Lövei, G., and Cartellieri, M. (2000). Ground beetles (Coleoptera, Carabidae) in forest fragments of the Manawatu, New Zealand: Collapsed assemblages? *Journal of Insect Conservation 4*:239-244.

Lövei, G. L., and Sunderland, K. D. (1996). Ecology and behaviour of ground beetles (Coleoptera: Carabidae). *Annual Review of Entomology 41*:231-256.

Luff, M. L. (1966). The abundance and diversity of the beetles fauna of grass tussocks. *Journal of Animal Ecology 35*:189-208.

Luff, M. L. (1975). Some features influencing the efficiency of pitfall traps. *Oecologia 19*:345-357.

Lunt, D. H., Zhang, D.-X., Szymura, J. M., and Hewitt, G. M. (1996). The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology 5*:153-65.

Maddison, D. R. (1994). Phylogenetic methods for infering the evolutionary history and processes of change in discreetly valued characters. *Annual Review of Entomology 39*:267-292.

Maddison, D. R., Baker, M. D., and Ober, K. A. (1999). Phylogeny of carabid beetles as inferred from 18S ribosomal DNA (Coleoptera: Carabidae). *Systematics Entomology 24*:103-138.

Maddison, W. P., and Maddison, D. R. (1992). *MacClade Version 3*. Sinauer Associates Inc, Sunderland.

Margush, T., and McMorris, F. R. (1981). Consensus n-trees. *Bulletin of Mathematics and Biology 43*:239-244.

Mayr, E. W. (1969). *Principles of systematics zoology*. McGraw-Hill Book Company, New York.

McGuinness, C. (2001). Threatened Carabid Beetle Recovery Plan (2001-2006) Draft. *Threatened Species Recovery Plan*:79.

Mitter, C. (1981). "Cladistics" in Botany. *Systematic Zoology 30*:373-376.

Miyamoto, M. M., and Fitch, W. M. (1995). Testing species phylogenies and phylogenetic methods with congruence. *Systematic Biology 44*:64-76.

Moeed, A., and Meads, M. J. (1985). Seasonality of pitfall trapped invertebrates in three types of native forest, Orongorongo Valley, New Zealand. *New Zealand Journal of Zoology 12*:17-53.

Molloy, J., and Davis, A. (1994). *Setting priorities for the conservation of New Zealand's threatened plants and animals, compiled by Christine Tisdall*, 2nd edition. Department of Conservation, Wellington.

Moritz, C., Dowling, T. E., and Brown, W. M. (1987). Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics 18*:269-92.

Nei, M., and Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press, Oxford.

Page, R. D. M., and Holmes, E. C. (1998). *Molecular evolution: A phylogenetic approach*. Blackwell Science Ltd, Oxford.

Pashley, D. P., and Ke, L. D. (1992). Sequence evolution in mitochondrial ribosomal and ND1 genes in lepidoptera: implications for phylogenetic analyses. *Molecular Biology and Evolution 9*: 1061-1075.

Patrick, B. H. (1982). Old Man Range trip. *The Weta 5*:37.

Patrick, B. H. (1997). Invertebrates of Macraes Ecological Distract. *Otago Conservancy Miscellaneous Series 30*:43.

Patrick, B. H., Barratt, B. I. P., Ward, J. B., and McLellan, I. D. (1993). Insects of the Waipori Ecological district, Lammerslaw Ecological Region. *Otago Conservancy Miscellaneous Series 16*:42.

Patterson, C., Williams, D. M., and Humphries, C. J. (1993). Congruence between molecular and morphological phylogenies. *Annual Review of Ecology and Systematics 24*:153-188.

Pawson, S. M., and Emberson, R. M. (2000). The conservation status of invertebrates in Canterbury. *Conservation Advisory Science Notes 320*:66.

Pawson, S. M., and Emberson, R. M. (2001). *Oregus inaequalis* Castelnau, its distribution, and abundance at Swampy Summit. *DOC Science Internal Series 6*:20.

Penny, D., Hendy, M. D., and Steel, M. A. (1992). Progress with methods for constructing evolutionary trees. *Trends in Ecology and Evolution 7*:73-79.

Platnick, N. I., Griswold, G. E., and Coddington, J. A. (1991). On missing entries in cladistic analysis. *Cladistics 7*:337-343.

Pohl, G. (1998). A morphological and genetic comparison of *Patrobus fossifrons* (Eschscholtz) and stygicus Chaudoir (Coleoptera: Carabidae). *Canadian Journal of Zoology 76*:689-703.

Posada, D., and Crandall, K. A. (1998). Model Test: testing the model of DNA substitution. *Bioinformatics 14*:817-818.

Pruser, F., and Mossakowski, D. (1998). Low substitution rates in mitochondrial DNA in Mediterranean carabid beetles. *Insect Molecular Biology 7*:121-128.

Putzeys, J. (1868). Les Broscides. *Stettiner entomologische Zeitung 29*:305-379.

Putzeys, J. (1873). Revision des Broscides de l'australie d'apres la collection de M le Comte de Castelnau. *Ann Mus Civ Nat giacomo Doria*:307-343.

Ramsay, G. W., Meads, M. J., Sherley, G. H., and Gibbs, G. W. (1988). Research on terrestrial insects in New Zealand. *Wildlife Research Liaison Group Research Review 10*:49.

Rand, D. M. (1994). Thermal habit, metabolic rate and the evolution mitochondrial DNA. *Trends in Ecology and Evolution 9*:125-130.

Ribera, I., Barraclough, T. G., and Vogler, A. P. (2001). The effect of habitat type on speciation rates and range movements in aquatic beetles: inferences from species-level phylogenies. *Molecular Ecology 10*:721-735.

Rodrigo, A. G., Kelly-Borges, M., Bergquist, P. R., and Bergquist, P. L. (1993). A randomisation test of the null hypothesis that two cladograms are sample estimates of a parametric phylogenetic tree. *New Zealand Journal of Botany 31*:257-268.

Roig-Junent, S. (2000). The subtribes and genera of the Tribe Broscini (Coleoptera: Carabidae): cladistics analysis, taxonomic treatment, and biogeographical considerations. *Bulletin of the American Museum of Natural History 255*:90.

Schoniger, M., and von Haeseler, A. (1995). Performance of the maximum likelihood, neighbour joining, and maximum parsimony methods when sequence sites are not independent. *Systematic Biology 44*:533-547.

Sequeira, A. S., Lanteri, A. A., Scataglinis, M. A., Confalonieris, V. A., and Farrell, B. D. (2000). Are flightless *Galapaganus* weevils older than the Galapagos Islands they inhabit? *Heredity 85*:20-29.

Sharpiro, A. M., and Porter, A. H. (1989). The lock-and-key hypothesis: Evolutionary and biosystematic interpretation of insect genitalia. *Annual Review of Entomology 34*:231-246.

Sharpiro, L. H. (1998). Hybridisation and geographic variation into meadow katydid contact zones. *Evolution 52*:784-796.

Shaw, A. J. (2000). Molecular phylogeography and cryptic speciation in the mosses, *Mielichhoferia elongata* and *M. mielichhoferiana*. *Molecular Ecology 9*:595.

Simon, C. (1991). Molecular systematics at the species boundary: Exploiting conserved and variable regions of mitochondrial genome of animals via direct sequencing from amplified DNA. Pages 33-71 In (G. M. Hewitt, Johnston, A. W. B., and Young, Y. P. W., eds.).*Molecular Techniques in Taxonomy* Springer-Verlag, Berlin.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America 87*:651-701.

Soltis, P. S., and Gitzendanner, M. A. (1999). Molecular systematics and the conservation of rare species. *Conservation Biology 13*:471-481.

Sota, T., and Vogler, A. P. (2001). Incongruence of mitochondrial and nuclear gene trees in the carabid beetles *Ohomopterus*. *Systematic Biology 50*:39-59.

Steel, M. A., Hendy, M. D., and Penny, D. (1993). Parsimony can be consistent. *Systematic Biology 42*:581-587.

Su, Z. H., Ohama, T., Okada, T., Nakamura, K., Ishikawa, R., and Osawa, S. (1996a). Phylogenetic relationships and evolution of the Japanese Carabinae ground beetles based on mitochondrial ND5 gene sequences. *Journal of Molecular Evolution 42*:124-129.

Su, Z. H., Tominaga, O., Ohama, T., Kajiwara, E., Ishikawa, R., Okada, T. S., *et al.* (1996b). Parallel evolution in radiation of *Ohomopterus* ground beetles inferred from mitochondrial ND5 gene sequences. *Journal of Molecular Evolution 43*:662-671.

Su, Z. H., Tominaga, O., Okamoto, M., and Osawa, S. (1998). Origin in diversification of hind-wingless *Damaster* ground beetles within the Japanese islands as deduced from mitochondrial ND5 gene sequences (Coleoptera, Carabidae). *Molecular Biology and Evolution 15*:1026-1039.

Sunnucks, P., and Hale, D. F. (1996). Numerous transposed sequences of mitochondrial Cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution 13*:510-24.

Swofford, D. L. (1998). *Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.* Sinauer Associates, Sunderland, Massachusetts.

Swofford, D. L., Olsen, G. J., Waddell, P. J., and Hillis, D. M. (1996). Phylogenetic Inference. Pages 407--514 In (D. M. Hillis, Moritz, C., and Mable, B. K., eds.). *Molecular Systematics* Sinauer Associates Inc, Sunderland.

Tennyson, A. (1998). Large carabid beetles Stephens Island 30 April- 3 May 1996. *Conservation Advisory Science Notes 172*:7.

Thiele, H. U. (1977). *Carabid beetles in their environment: A study on habitat selection by adaptations to physiology and behaviour.* Springer-Verlag, Berlin.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research 25*:4876-4882.

Topping, C. J., and Sunderland, K. D. (1992). Limitations to the use of pitfall traps in ecological studies exemplified by a study of spiders in a field of winter wheat. *Journal of Applied Ecology 29*:485-491.

Trewick, S. A. (2000). Mitochondrial DNA sequences support allozyme evidence for cryptic radiation of New Zealand *Peripatoides* (Onychophora). *Molecular Ecology 9*:269.

Vogler, A. P., and Desalle, R. (1994). Evolution and phylogenetic information content of the ITS-1 region in the tiger beetle *Cincindela dorsalis*. *Molecular Biology and Evolution 11*:393-405.

Vogler, A. P., Knisley, C. B., Glueck, S. B., Hill, J. M., and Desalle, R. (1993). Using molecular and ecological data to diagnose endangered populations of the puritan tiger beetle *Cincindela puritana*. *Molecular Ecology 2*:375-383.

White, A. (1846). Insects of New Zealand. Pages 1-27 *Zoology of the voyage of the HMS Erebus and Terror.*

Yang, Z. (1994). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *Journal of Molecular Evolution 39*:306-314.

Yang, Z., Goldman, N., and Friday, A. (1994). Comparison of models for nucleotide substitution used in maximum-likelihood phylogenetic estimation. *Molecular Biology and Evolution 11*:316-324.

Zaidi, R. H., Jaal, Z., Hawkes, N. J., Hemingway, J., and Symondson, W. O. C. (1999). Can multiple-copy sequences of prey DNA be detected amongst the doubt contents of invertebrate predators? *Molecular Ecology 8*:2081-2087.

Zhang, D.-X., and Hewitt, G. M. (1996a). Assessment of the universality and utility of a set of conserved mitochondrial COI primers in insects. *Insect molecular Biology 6*:143-50.

Zhang, D.-X., and Hewitt, G. M. (1996b). Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution 11*:247-251.

Zhang, D.-X., and Hewitt, G. M. (1997). Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies. *Biochemical Systematics and Ecology 25*:99-120.

Appendix A Morphological Character States

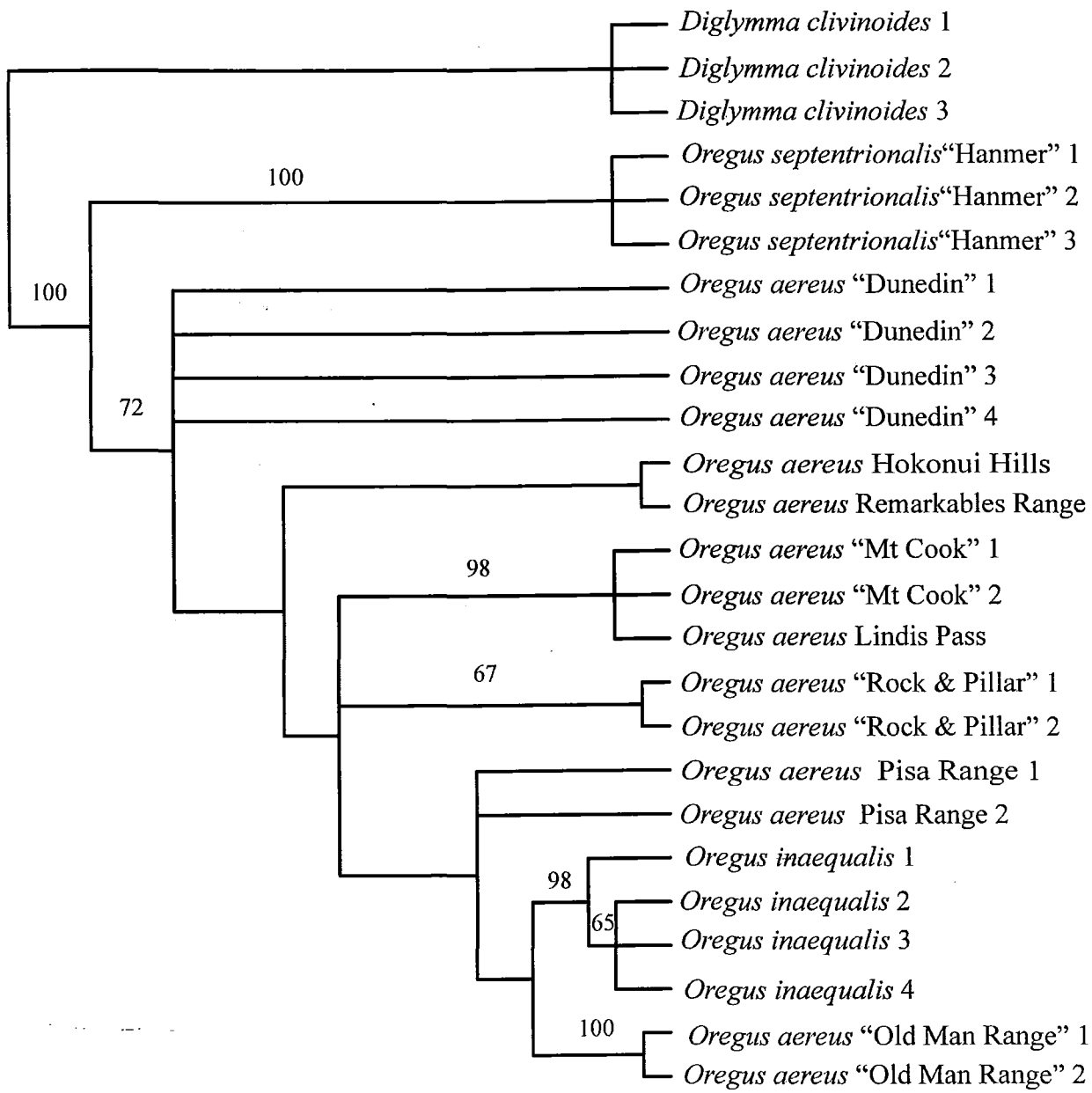
Character states are shown in **bold** font.

Character list

1. Number of pairs of supraorbital setae including setae occurring in transverse shallow depression of the vertex (Figure 7A). **0**, zero; **1**, one; **2**, two; **3**, three; **4**, four; **5**, five; **6**,six.
2. Colour of femora and tibia in adult individuals. **0**, black; **1**, red-brown.
3. Number of setiferous punctures on elytral margin anterior to the transverse metasternal suture. **0**, three; **1**, four; **2**, five.
4. Number of setiferous punctures on margin of elytra between the transverse metasternal suture and the apical margin of the second ventrite. **0**, two; **1**, three; **2**, four.
5. Number of setiferous punctures on margin of elytra from the third ventrite to the base of the elytra.**0**, five;**1**, six;**2**, seven; **3**, eight; **4**, nine; **5**, twelve.
6. Shape of antenna **0**, moniliform; **1**, filiform.
7. Excluding setae of the apical margin, ventrite 6 has setiferous punctures either side of the midline. **0**, zero; **1**, one; **2**, two.
8. The posterior lateral depressions of the pronotum are. **0**, distinct; **1**, faint or non-existent.
9. Lateral depressions of the clypeus are. **0**, distinct; **1**, faint, difficult to discern even with the aid of a microscope.
10. Form of labral margin. **0**, labrum distinctly emarginate; **1**, labrum with visible shallow curvature; **2**, labral margin linear with no visible concavity to the margin .
11. Location of setation on the left paramere of the male genitalia. **0**, on an apical projection of the paramere; **1**, no setae; **2**, on edge of paramere that does not have an (or has a very reduced) apical projection; **3**, on the edge of the paramere and on a distinct apical projection.
12. Apical portion of the left paramere of the male genitalia forms. **0**, a long apical projection; **1**, a short apical projection; **2**, no apical projection and the paramere is rounded at the tip.
13. Transverse distance across the tip of the aedeagus is. **0**, < 0.2mm; **1**, 0.2-0.29mm; **2**, 0.30-0.45mm; **3**, 0.45mm.
14. The apical plate (originally described by Ball (1956)) is folded within sclerite y when the internal sac is in repose. The apical plate has. **0**, no sclerotised projections; **1**, one sclerotised projection (Figure 2.6C); **2**, two sclerotised projections (Figure 2.6B); **3**, three sclerotised projections.
15. Basal projection of the apical plate associated with the internal sac of the aedeagus is. **0**, absent; **1**, distinctly thickened, or spatulate; **2**, thin, highly pointed and less sclerotised.
16. Tip of the basal process of the apical plate associated with the internal sac. **0**, absent; **1**, extended to the right (Figure 2.6A); **2**, not extended laterally in anyway.
17. The right paramere of the male genitalia is highly setiferous on its ventral surface. Setae extend. **0**, the full length of the right paramere; **1**, three-quarters the length; **2**, two-thirds the length; **3**, half the length; **4**, one quarter the length; **5**, one-third the length.
18. Sclerite x of Ball (1956) is. **0**, short and thick; **1**, long and thin.
19. Sclerite y of Ball (1956) is shaped. **0**, approximately like a y with two proximal points and is heavily sclerotised; **1**, more like half a globe and weakly sclerotised.

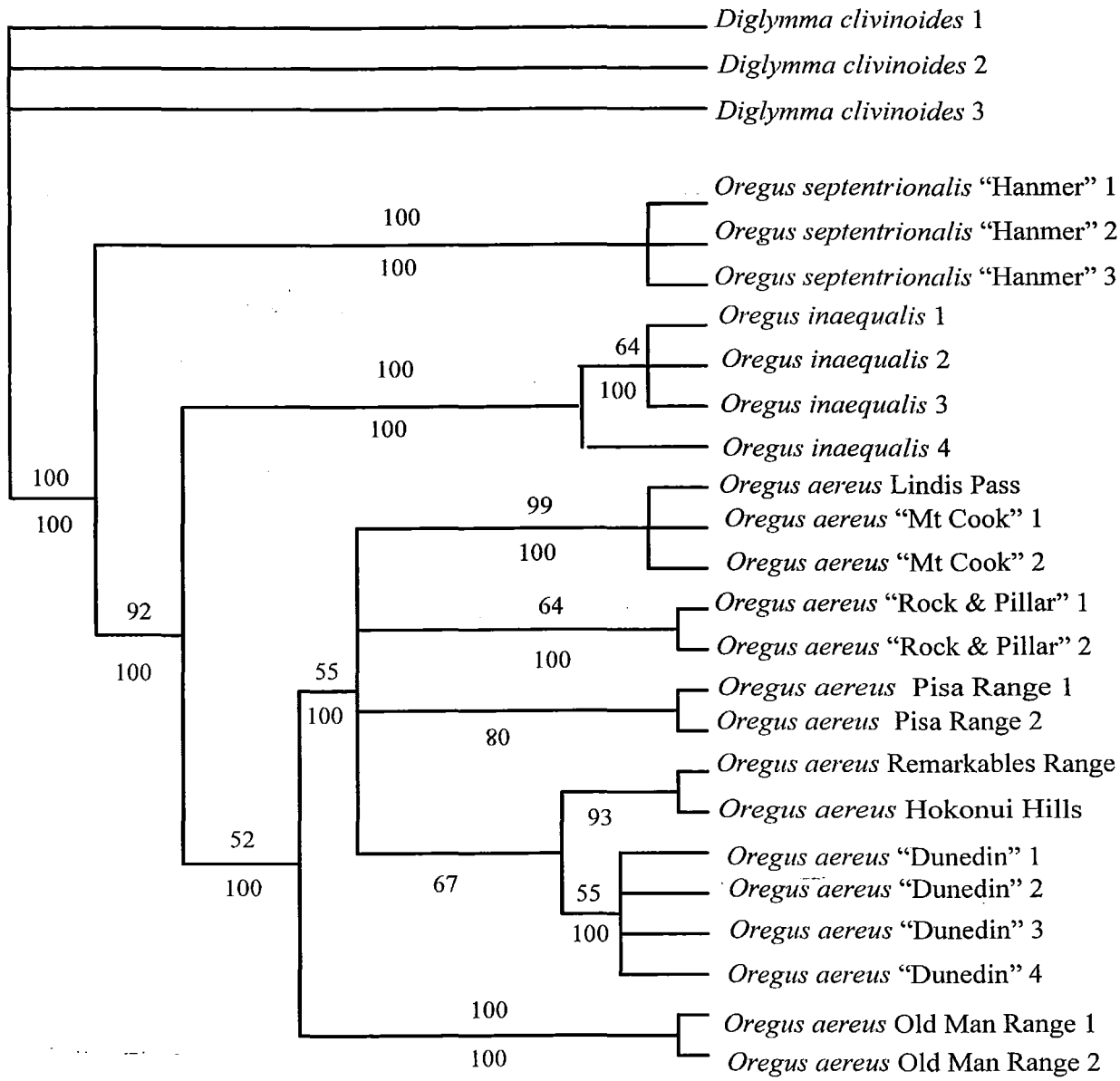
20. The apical plate is covered in many small hairs, hairs are. **0**, short and dark; **1**, long and dark; **2**, apical plate is very sparsely covered by hairs.
21. In some populations there is a defined bump at the base of the aedeagus on the left-hand side (from posterior ventral view), which acts as an attachment point for the condyle that links the aedeagus with the left paramere. **0**, structure not present; **1**, structure present.
22. In some populations there is a defined bump at the base of the aedeagus on the right-hand side (from posterior ventral view) this acts as an attachment point for the condyle that links the aedeagus with the right paramere. **0**, structure present; **1**, structure present.
23. Shape of the tip of the aedeagus is. **0**, rounded (Figure 2.4A-B); **1**, blunt (Figure 2.4D); **2**, distinctly enlarged (Figure 2.4C).
24. Ventral surface of the aedeagus is smooth and highly sclerotised, and some cases there may be a distinct, though small, notch close to the tip (Figure 2.4B). **0**, notch absent; **1**; notch present.
25. Spermatheca of adult female genitalia is. **0**, unsclerotised milky white structure; **1**, lightly sclerotised, visible with acid fuchsia stain; **2**, heavily sclerotised.
26. Spermatheca of adult female genitalia is. **0**, short and thickened in the apical three- quarters; **1**, long and thin with no obvious thickening; **2**, long and thick.
27. Basal compartment of the accessory gland of the female genitalia (Figure 2.5D) is. **0**, not sclerotised; **1**, lightly sclerotised; **2**, heavily sclerotised.

Appendix B COI Maximum Likelihood Tree



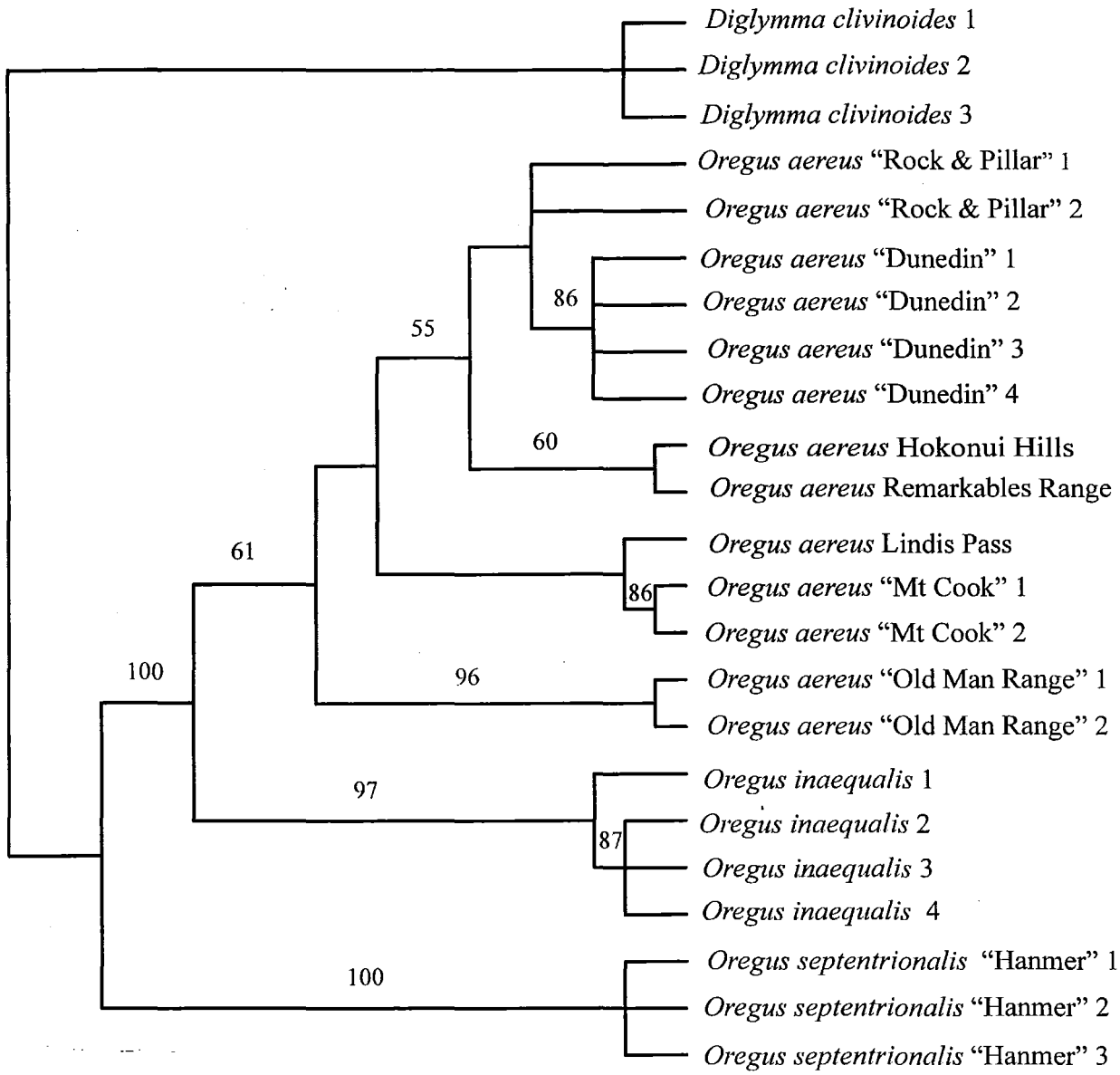
Phylogenetic tree inferred from COI, using maximum likelihood (TVM+G model of evolution) ln=1669.59825. Values above branches represent bootstrap support from 1000 replicates.

Appendix C COI Parismony Tree



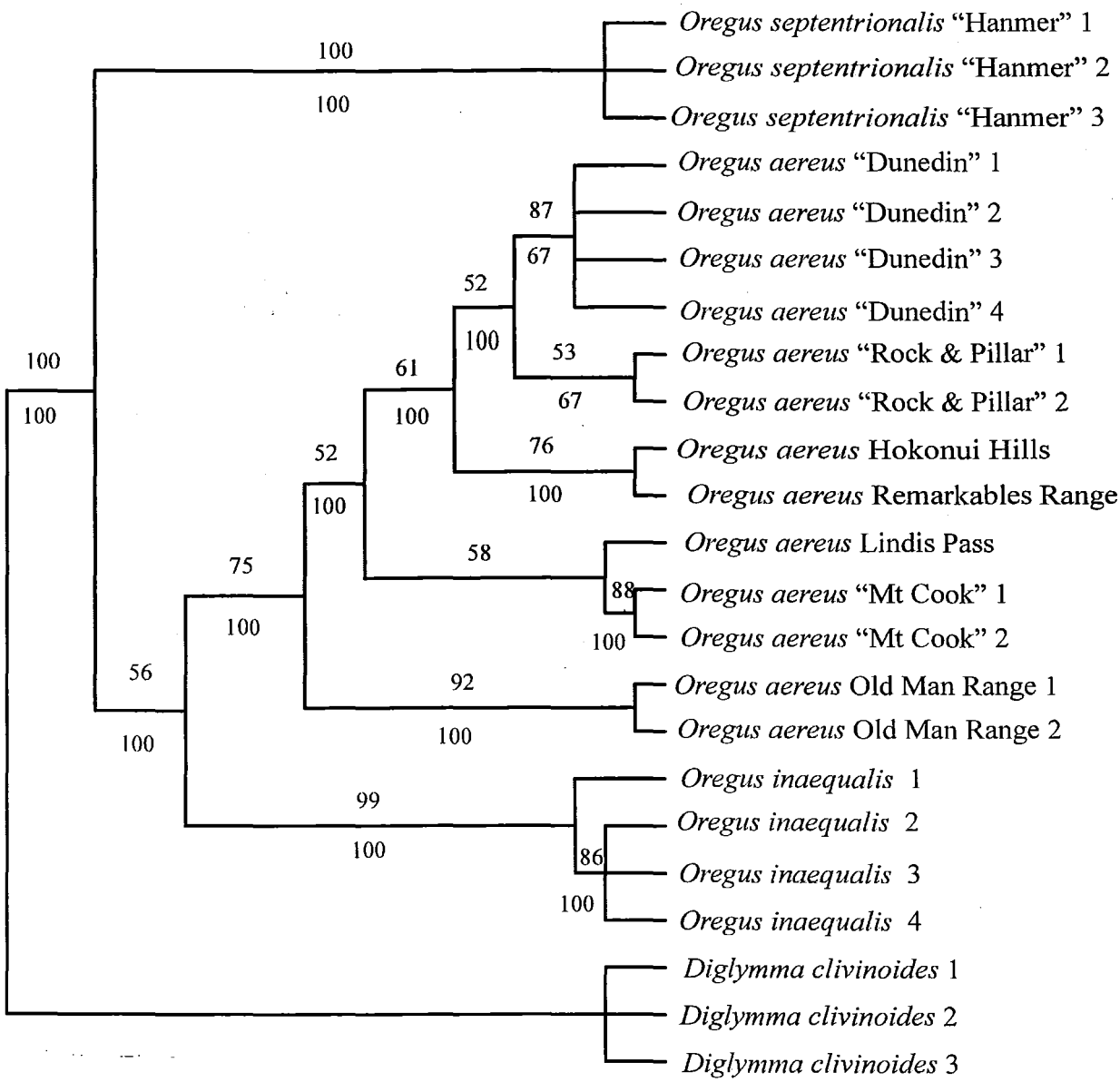
Majority rule consensus tree of 15 equally parsimonious trees (Length 139 steps, CI= 0.90, RI= 0.96), inferred from the CO I gene region. Majority rule consensus values are shown below branches and bootstrap support from 1000 replicates is shown above.

Appendix D ND1 Maximum Likelihood Tree



Phylogenetic tree of the ND1 gene region inferred using maximum likelihood (TIM model of evolution), ln=906.08. Values above the branches indicate bootstrap support from 1000 replicates.

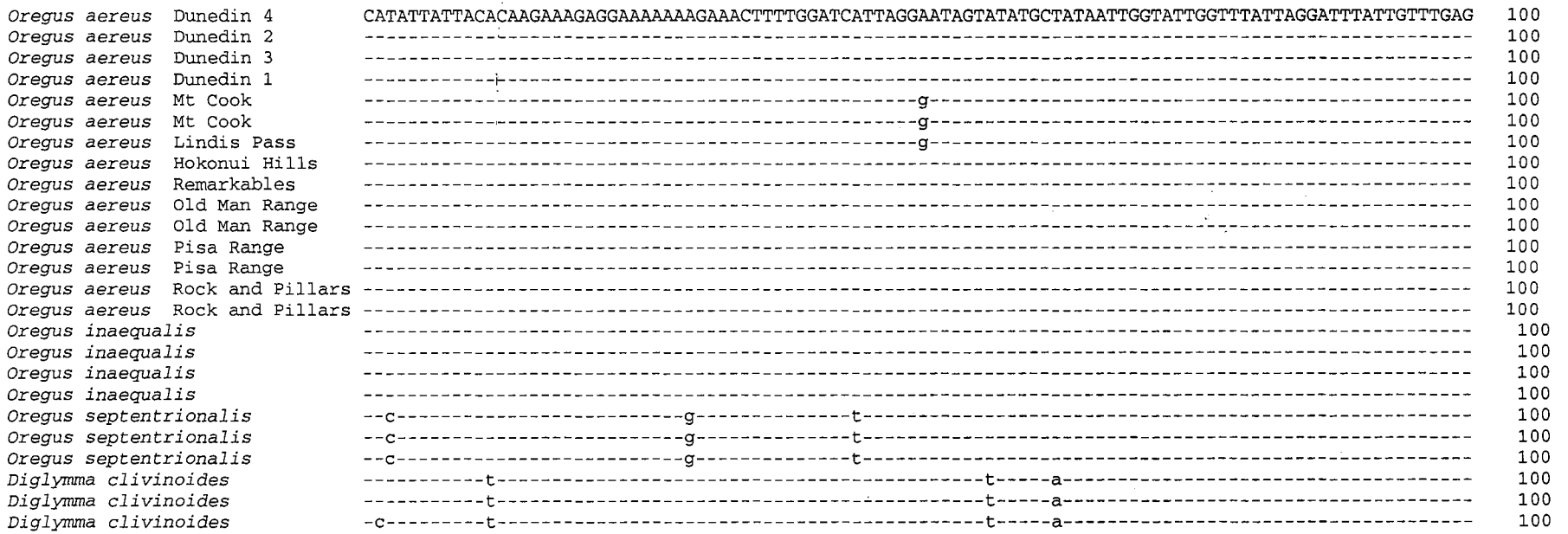
Appendix E ND1 Parsimony Tree

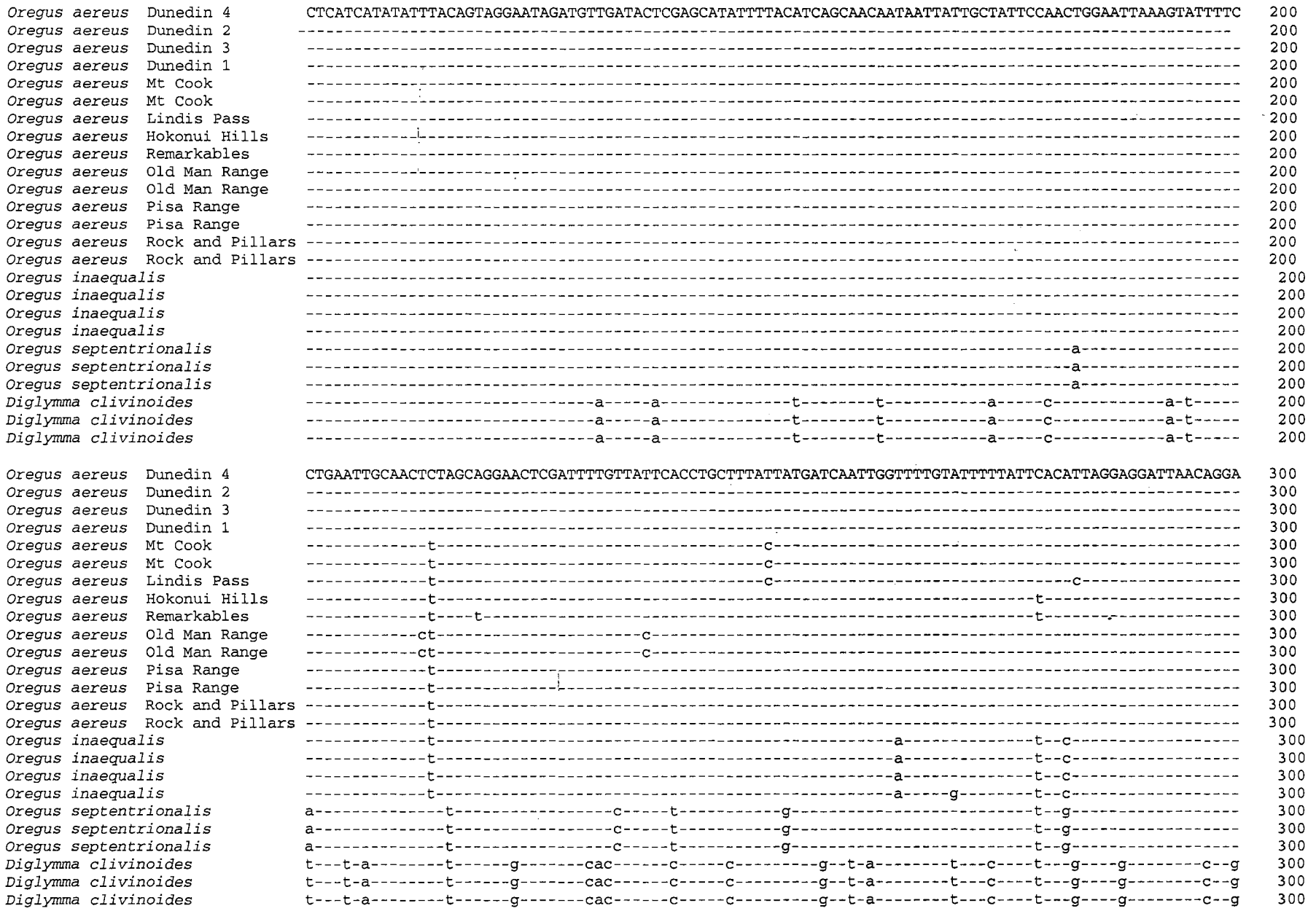


Majority rule consensus tree of three equally parsimony trees (Length 72 steps, CI= 0.89, RI= 0.96) inferred from the ND1 gene region. Numbers below the branches indicate majority rule consensus values, numbers above branches indicate bootstrap support from 1000 replictes.

Appendix F mtDNA Sequences

COI Gene Region




Oregus aereus Dunedin 4 CTCATCATATATTTACAGTAGGAATAGATGTTGATACTCGAGCATATTTTACATCAGCAACAATAATTATTGCTATTCCAACTGGAATTAAAGTATTTTC 200
Oregus aereus Dunedin 2 200
Oregus aereus Dunedin 3 200
Oregus aereus Dunedin 1 200
Oregus aereus Mt Cook 200
Oregus aereus Mt Cook 200
Oregus aereus Lindis Pass 200
Oregus aereus Hokonui Hills 200
Oregus aereus Remarkables 200
Oregus aereus Old Man Range 200
Oregus aereus Old Man Range 200
Oregus aereus Pisa Range 200
Oregus aereus Pisa Range 200
Oregus aereus Rock and Pillars 200
Oregus aereus Rock and Pillars 200
Oregus inaequalis 200
Oregus inaequalis 200
Oregus inaequalis 200
Oregus inaequalis 200
Oregus septentrionalis 200
Oregus septentrionalis 200
Oregus septentrionalis 200
Diglymma clivinoides 200
Diglymma clivinoides 200
Diglymma clivinoides 200
Oregus aereus Dunedin 4 CTGAATTGCAACTCTAGCAGGAACTCGATTTTGTTATTCACCTGCTTTATTATGATCAATTGGTTTTGTATTTTTATTCACATTAGGAGGATTAACAGGA 300
Oregus aereus Dunedin 2 300
Oregus aereus Dunedin 3 300
Oregus aereus Dunedin 1 300
Oregus aereus Mt Cook 300
Oregus aereus Mt Cook 300
Oregus aereus Lindis Pass 300
Oregus aereus Hokonui Hills 300
Oregus aereus Remarkables 300
Oregus aereus Old Man Range 300
Oregus aereus Old Man Range 300
Oregus aereus Pisa Range 300
Oregus aereus Pisa Range 300
Oregus aereus Rock and Pillars 300
Oregus aereus Rock and Pillars 300
Oregus inaequalis 300
Oregus inaequalis 300
Oregus inaequalis 300
Oregus inaequalis 300
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Oregus septentrionalis 300
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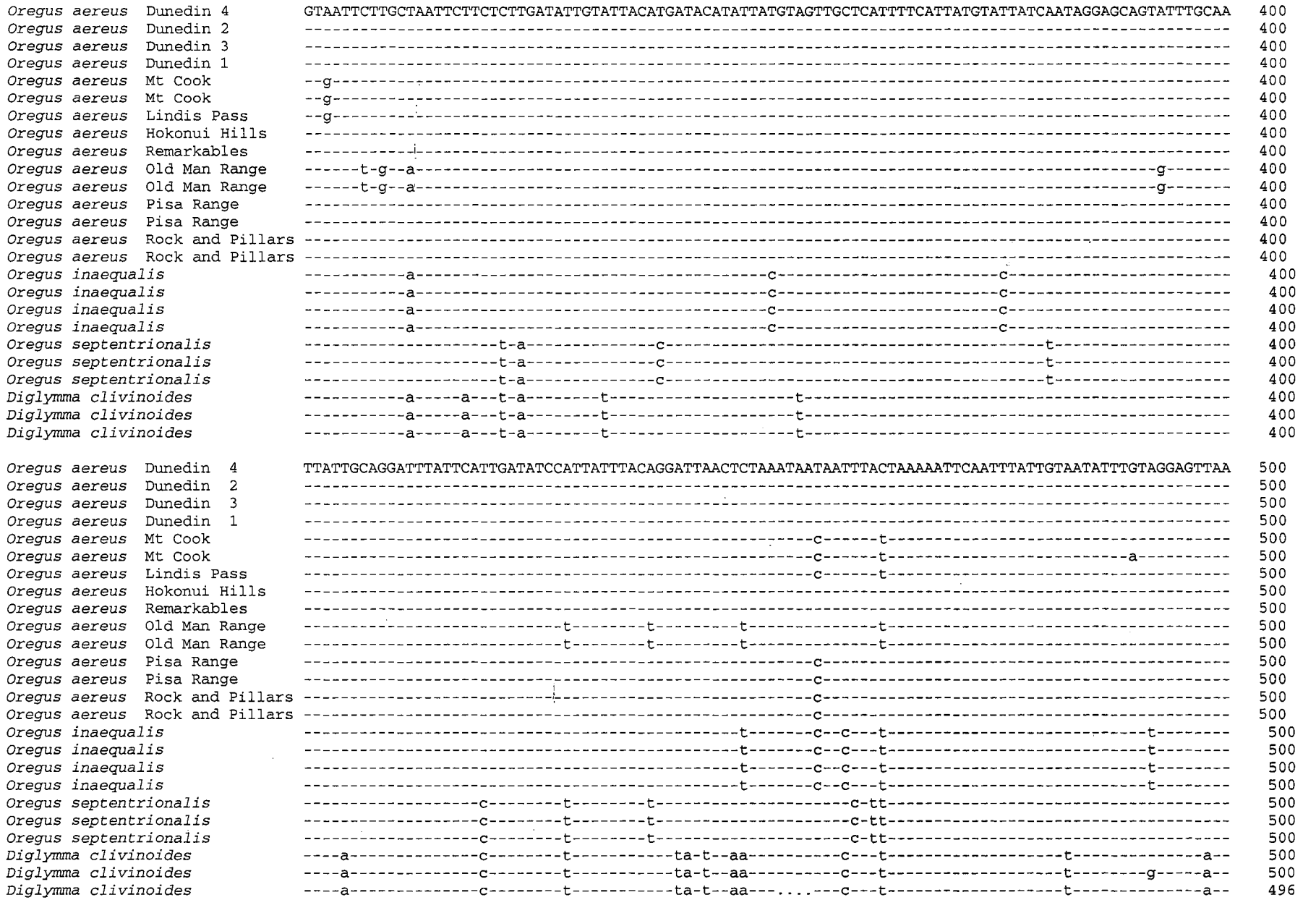
Diglymma clivinoides 300
Diglymma clivinoides 300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Oregus aereus Dunedin 3 400
Oregus aereus Dunedin 1 400
Oregus aereus Mt Cook 400
Oregus aereus Mt Cook 400
Oregus aereus Lindis Pass 400
Oregus aereus Hokonui Hills 400
Oregus aereus Remarkables 400
Oregus aereus Old Man Range 400
Oregus aereus Old Man Range 400
Oregus aereus Pisa Range 400
Oregus aereus Pisa Range 400
Oregus aereus Rock and Pillars 400
Oregus aereus Rock and Pillars 400
Oregus inaequalis 400
Oregus inaequalis 400
Oregus inaequalis 400
Oregus inaequalis 400
Oregus septentrionalis 400
Oregus septentrionalis 400
Oregus septentrionalis 400
Diglymma clivinoides 400
Diglymma clivinoides 400
Diglymma clivinoides 400
Oregus aereus Dunedin 4 TTATTGCAGGATTTATTCATTGATATCCATTATTTACAGGATTAACTCTAAATAATAATTTACTAAAAATTCAATTTATTGTAATATTTGTAGGAGTTAA 500
Oregus aereus Dunedin 2 500
Oregus aereus Dunedin 3 500
Oregus aereus Dunedin 1 500
Oregus aereus Mt Cook 500
Oregus aereus Mt Cook 500
Oregus aereus Lindis Pass 500
Oregus aereus Hokonui Hills 500
Oregus aereus Remarkables 500
Oregus aereus Old Man Range 500
Oregus aereus Old Man Range 500
Oregus aereus Pisa Range 500
Oregus aereus Pisa Range 500
Oregus aereus Rock and Pillars 500
Oregus aereus Rock and Pillars 500
Oregus inaequalis 500
Oregus inaequalis 500
Oregus inaequalis 500
Oregus inaequalis 500
Oregus septentrionalis 500
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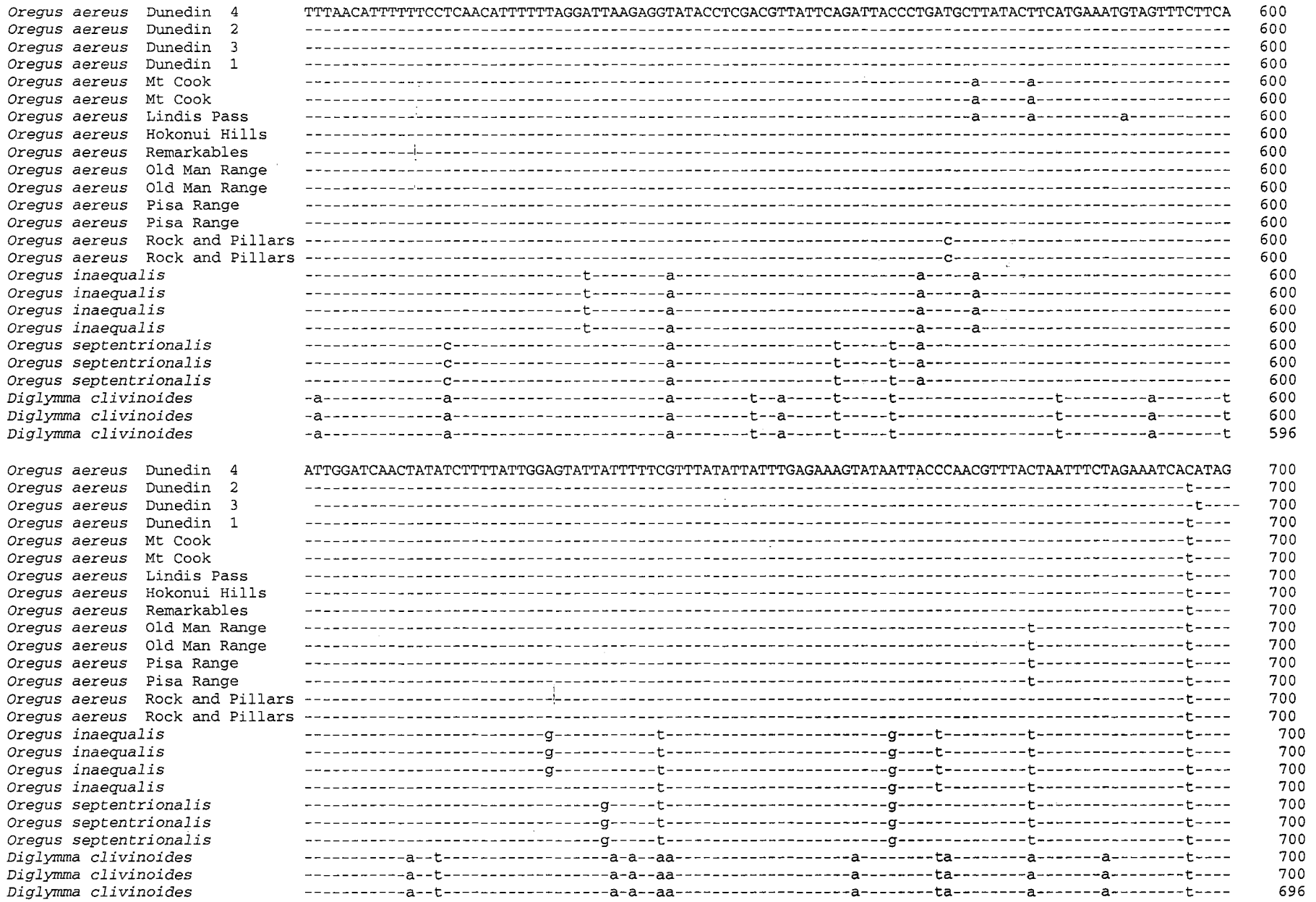
Diglymma clivinoides 500
Diglymma clivinoides 500
Diglymma clivinoides 496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Oregus aereus Dunedin 2
Oregus aereus Dunedin 3
Oregus aereus Dunedin 1
Oregus aereus Mt Cook
Oregus aereus Mt Cook
Oregus aereus Lindis Pass
Oregus aereus Hokonui Hills
Oregus aereus Remarkables
Oregus aereus Old Man Range
Oregus aereus Old Man Range
Oregus aereus Pisa Range
Oregus aereus Pisa Range
Oregus aereus Rock and Pillars
Oregus aereus Rock and Pillars
Oregus inaequalis
Oregus inaequalis
Oregus inaequalis
Oregus inaequalis
Oregus septentrionalis
Oregus septentrionalis
Oregus septentrionalis
Diglymma clivinoides
Diglymma clivinoides
Diglymma clivinoides
TTTAACATTTTTTCCTCAACATTTTTTAGGATTAAGAGGTATACCTCGACGTTATTCAGATTACCCTGATGCTTATACTTCATGAAATGTAGTTTCTTCA 600
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ATTGGATCAACTATATCTTTTATTGGAGTATTATTTTTCGTTTATATTATTTGAGAAAGTATAATTACCCAACGTTTACTAATTTCTAGAAATCACATAG 700
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696

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Oregus aereus  Dunedin  4       AAACTTCAATTG.AATGATTTCAAAAATATCCTCCTGCTGAA 741
Oregus aereus  Dunedin  2       ------------.------------------------------ 741
Oregus aereus  Dunedin  3       ------------.------------------------------ 741
Oregus aereus  Dunedin  1       ------------.------------------------------ 741
Oregus aereus  Mt Cook          ------------.------------------------------ 741
Oregus aereus  Mt Cook          ------------.------------------------------ 741
Oregus aereus  Lindis Pass      ------------.------------------------------ 741
Oregus aereus  Hokonui Hills    ------------g----------------------------- 742
Oregus aereus  Remarkables      ------------.------------------------------ 741
Oregus aereus  Old Man Range    ------------.------------------------------ 741
Oregus aereus  Old Man Range    ------------.------------------------------ 741
Oregus aereus  Pisa Range       ------------.------------------------------ 741
Oregus aereus  Pisa Range       ------------.--------------------------     741
Oregus aereus  Rock and Pillars ------------.------------------------------ 741
Oregus aereus  Rock and Pillars ------------.------------------------------ 741
Oregus inaequalis               -----------.-------------------------       741
Oregus inaequalis               ------------.------------------------------ 741
Oregus inaequalis               ------------.------------------------------ 741
Oregus inaequalis               ------------.------------------------------ 741
Oregus septentrionalis          ----a-------.------------------------------ 741
Oregus septentrionalis          ----a-------.------------------------------ 741
Oregus septentrionalis          ----a-------.------------------------------ 741
Diglymma clivinoides            ----c--t----.-------a-------t-------t----- 741
Diglymma clivinoides            ----c--t----.-------a-------t-------t----- 741
Diglymma clivinoides            ----c--t----.-------a-------t-------t----- 741
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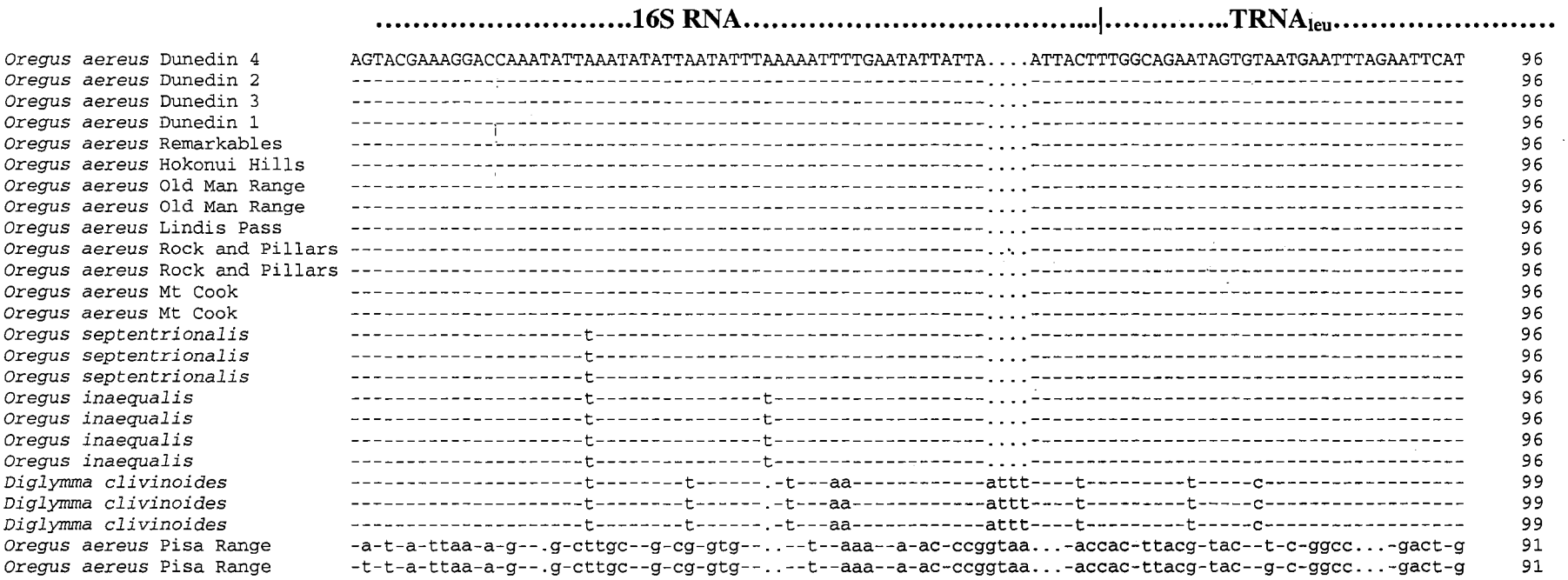
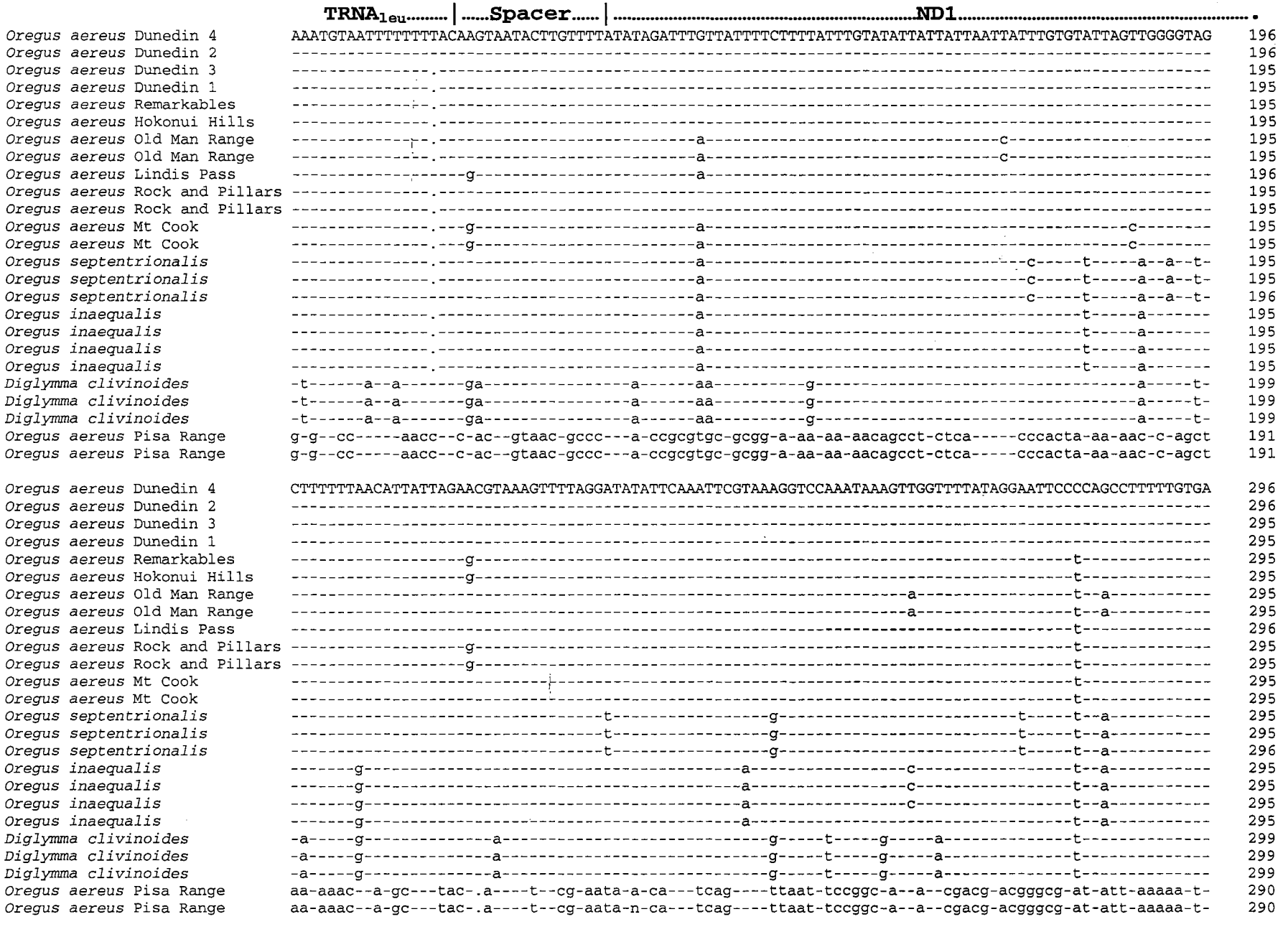
ND1 Gene Region




TRNAleu
Spacer
ND1
Oregus aereus Dunedin 4
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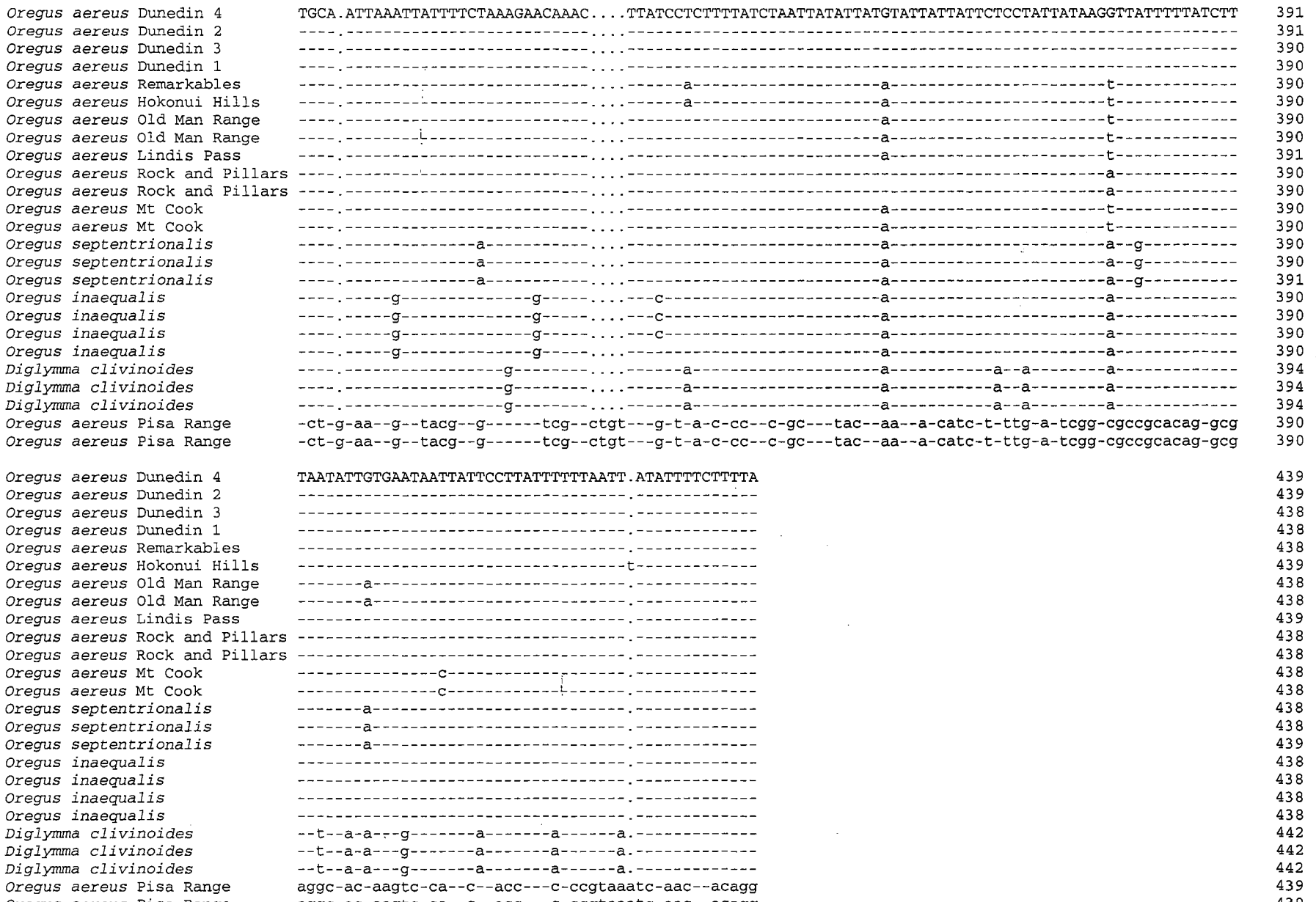
Oregus aereus Dunedin 3
Oregus aereus Dunedin 1
Oregus aereus Remarkables
Oregus aereus Hokonui Hills
Oregus aereus Old Man Range
Oregus aereus Old Man Range
Oregus aereus Lindis Pass
Oregus aereus Rock and Pillars
Oregus aereus Rock and Pillars
Oregus aereus Mt Cook
Oregus aereus Mt Cook
Oregus septentrionalis
Oregus septentrionalis
Oregus septentrionalis
Oregus inaequalis
Oregus inaequalis
Oregus inaequalis
Oregus inaequalis
Diglymma clivinoides
Diglymma clivinoides
Diglymma clivinoides
Oregus aereus Pisa Range
Oregus aereus Pisa Range
Oregus aereus Dunedin 4
Oregus aereus Dunedin 2
Oregus aereus Dunedin 3
Oregus aereus Dunedin 1
Oregus aereus Remarkables
Oregus aereus Hokonui Hills
Oregus aereus Old Man Range
Oregus aereus Old Man Range
Oregus aereus Lindis Pass
Oregus aereus Rock and Pillars
Oregus aereus Rock and Pillars
Oregus aereus Mt Cook
Oregus aereus Mt Cook
Oregus septentrionalis
Oregus septentrionalis
Oregus septentrionalis
Oregus inaequalis
Oregus inaequalis
Oregus inaequalis
Oregus inaequalis
Diglymma clivinoides
Diglymma clivinoides
Diglymma clivinoides
Oregus aereus Pisa Range
Oregus aereus Pisa Range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Oregus aereus Dunedin 2 ----.----------....---------- 391
Oregus aereus Dunedin 3 ----.----------....---------- 390
Oregus aereus Dunedin 1 ----.----------....---------- 390
Oregus aereus Remarkables ----.----------....------a----------a----------t---------- 390
Oregus aereus Hokonui Hills ----.----------....------a----------a----------t---------- 390
Oregus aereus Old Man Range ----.----------....----------a----------t---------- 390
Oregus aereus Old Man Range ----.----------....----------a----------t---------- 390
Oregus aereus Lindis Pass ----.----------....----------a----------t---------- 391
Oregus aereus Rock and Pillars ----.----------....----------a---------- 390
Oregus aereus Rock and Pillars ----.----------....----------a---------- 390
Oregus aereus Mt Cook ----.----------....----------a----------t---------- 390
Oregus aereus Mt Cook ----.----------....----------a----------t---------- 390
Oregus septentrionalis ----.----------a----------....----------a----------a--g---------- 390
Oregus septentrionalis ----.----------a----------....----------a----------a--g---------- 390
Oregus septentrionalis ----.----------a----------....----------a----------a--g---------- 391
Oregus inaequalis ----.-----g----------g-----...---c----------a----------a---------- 390
Oregus inaequalis ----.-----g----------g-----...---c----------a----------a---------- 390
Oregus inaequalis ----.-----g----------g-----...---c----------a----------a---------- 390
Oregus inaequalis ----.-----g----------g-----...----------a----------a---------- 390
Diglymma clivinoides ----.----------g--------...------a----------a----------a--a--------a---------- 394
Diglymma clivinoides ----.----------g--------...------a----------a----------a--a--------a---------- 394
Diglymma clivinoides ----.----------g--------...------a----------a----------a--a--------a---------- 394
Oregus aereus Pisa Range -ct-g-aa--g--tacg--g------tcg--ctgt---g-t-a-c-cc--c-gc---tac--aa--a-catc-t-ttg-a-tcgg-cgccgcacag-gcg 390
Oregus aereus Pisa Range -ct-g-aa--g--tacg--g------tcg--ctgt---g-t-a-c-cc--c-gc---tac--aa--a-catc-t-ttg-a-tcgg-cgccgcacag-gcg 390
Oregus aereus Dunedin 4 TAATATTGTGAATAATTATTCCTTATTTTTTAATT.ATATTTTCTTTTA 439
Oregus aereus Dunedin 2 ----------.---------- 439
Oregus aereus Dunedin 3 ----------.---------- 438
Oregus aereus Dunedin 1 ----------.---------- 438
Oregus aereus Remarkables ----------.---------- 438
Oregus aereus Hokonui Hills ----------t---------- 439
Oregus aereus Old Man Range -------a----------.---------- 438
Oregus aereus Old Man Range -------a----------.---------- 438
Oregus aereus Lindis Pass ----------.---------- 439
Oregus aereus Rock and Pillars ----------.---------- 438
Oregus aereus Rock and Pillars ----------.---------- 438
Oregus aereus Mt Cook ----------c----------.---------- 438
Oregus aereus Mt Cook ----------c----------.---------- 438
Oregus septentrionalis -------a----------.---------- 438
Oregus septentrionalis -------a----------.---------- 438
Oregus septentrionalis -------a----------.---------- 439
Oregus inaequalis ----------.---------- 438
Oregus inaequalis ----------.---------- 438
Oregus inaequalis ----------.---------- 438
Oregus inaequalis ----------.---------- 438
Diglymma clivinoides --t--a-a---g-------a-------a------a.---------- 442
Diglymma clivinoides --t--a-a---g-------a-------a------a.---------- 442
Diglymma clivinoides --t--a-a---g-------a-------a------a.---------- 442
Oregus aereus Pisa Range aggc-ac-aagtc-ca--c--acc---c-ccgtaaatc-aac--acagg 439
Oregus aereus Pisa Range aggc-ac-aagtc-ca--c--acc---c-ccgtaaatc-aac--acagg 439

Appendix G Collections of *O. inaequalis*

Known specimens of *Oregus inaequalis* in collections; nationally, internationally and privately that have been observed by S.M. Pawson.

Location	Sex	Date	Collector	Collection
Dunedin	1M, 3?	?.?.1863	Castelnau	Museo Civico Di Storia Naturale G. Doria Genova
Port Chalmers	1?	?.x.1901	J.J. Walker	BMNH
Port Chalmers	2?	?.ix.1902	J.J.Walker	Hudson Collection, National Museum
Waitati	1?	11.xi.1923	C.E.Clarke	AMNZ
Waitati	1M	14.x.1923	C.E.Clarke	AMNZ
Waitati	3F, 1M , 3?	7.x.1923	C.E.Clarke	AMNZ
Waitati	2?	7.x.1923	C.E.Clarke	National Museum
Waitati	1F	18.x.1925	C.E.Clarke	AMNZ
Waitati	4?	18.ix.1926	C.E.Clarke	AMNZ
Waitati	2?	18.ix.1926	C.E. Clarke Collection, BMNH	C.E. Clarke Collection, BMNH
Waitati Hills	1?	12.ix.1926	C.E.Clarke	A.E.Brookes Collection, NZAC
?	1F	?.1.1934	von Staudinger	F. van Emden Collection, BMNH
Mihiwaka, nr Port Chalmers	1?	21.i.1947	C.E. Clarke Collection, BMNH	C.E. Clarke Collection, BMNH
Leith Valley	2?	18.iv.1960	I. Townsend	I.Townsend
Leith Saddle	1?	24.ix.1966	R.R. Forster	Otago Museum(W)
Leith Saddle	1?	5.xi.1967	R.R. Forster	Otago Museum(W)
Leith Saddle	1?	2.x11.1967	R.R. Forster	Otago Museum(W)
Leith Saddle	1?	17.vi.1967	R.R. Forster	Otago Museum(W)
Ross Ck Reservoir	1?	18.x.1981	J.C.Watt	NZAC
Mt Cargill	1?	21.x.1981	J.C.Watt	NZAC
Swampy Summit	1?	16.xii.1984	B.I.P.Barratt	I. Townsend
Swampy Summit	1?	17.xi.1984	B.I.P. Barratt	LUNZ
Swampy Summit	11?	17.xi.-16.xii.1984	B.I.P. Barratt	B.I.P. Barratt
Swampy Summit	10?	16.xii.1984-12.i.1985	B.I.P. Barratt	B.I.P. Barratt
Swampy Summit	8?	12.i.1985-16.ii.1985	B.I.P. Barratt	B.I.P. Barratt
Leith Saddle	1F	25.xii.1989	A.C.Harris	Otago Museum
Bush track Swampy Summit	1?	26.xi.1993	I. Townsend	I.Townsend
Swampy Summit	1M, 1?	11.ii.1995	B and H. Patrick	Otago Museum
Swampy Summit	1F	23.xi.1997	E.Edwards, B and H. Patrick	E. Edwards
Flagstaff Hill	1M	?	?	A.E.Brookes Collection, NZAC
? Flagstaff Hill	1?	?	?	A.E.Brookes Collection, NZAC
Port Chalmers	1?	?	?	Hutton Collection, CMNZ
?	1?	?	?	CMNZ
?	1F	?	Wakefield	CMNZ
?	1?	?	?	Pasco Collection, BMNH

Appendix H Pitfall trap design

Photo of pitfall trap and rain cover used for this study.