

# Morphological and molecular analysis of the carabid genus *Oregus*, with identification of two new species

DOC SCIENCE INTERNAL SERIES 94

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Published by  
Department of Conservation  
P.O. Box 10-420  
Wellington, New Zealand

*DOC Science Internal Series* is a published record of scientific research carried out, or advice given, by Department of Conservation staff, or external contractors funded by DOC. It comprises progress reports and short communications that are generally peer-reviewed within DOC, but not always externally refereed. Fully refereed contract reports funded from the Conservation Services Levy are also included.

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© January 2003, New Zealand Department of Conservation

ISSN 1175-6519

ISBN 0-478-22361-7

This is a client report commissioned by Otago Conservancy and funded from the Unprogrammed Science Advice fund. It was prepared for publication by DOC Science Publishing, Science & Research Unit; editing and layout by Geoff Gregory. Publication was approved by the Manager, Science & Research Unit, Science Technology and Information Services, Department of Conservation, Wellington.

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# Morphological and molecular analysis of the carabid genus *Oregus*, with identification of two new species

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## ABSTRACT

A thorough revision of the New Zealand carabid genus *Oregus* Putzeys, 1868 was undertaken. All known and available specimens (2196) from national and selected international collections were examined. Both adult morphology (genitalic and external) and DNA sequence data (partial NADH dehydrogenase 1 and cytochrome oxidase I gene regions) were analysed to estimate the phylogeny. Phylogenetic results indicate that the genus comprises four species, including two new species from Marlborough and North Canterbury, assigned the manuscript names *Oregus* “septentrionalis” and *Oregus* “crypticus”, respectively. No new species were found from the greater Dunedin area as had been previously suggested. Formal taxonomic descriptions are not provided, as these will be published at a later stage.

Keywords: Carabidae, *Oregus* spp., taxonomy, phylogeny, morphology.

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Pawson, S.M; Emberson, R.M.; Armstrong, K.F.; Paterson, A.M. 2003: Morphological and molecular analysis of the carabid genus *Oregus*, with identification of two new species. *DOC Science Internal Series 94*. Department of Conservation, Wellington. 11 p.

# 1. Introduction

Putzeys (1868) first described the carabid genus *Oregus* Putzeys (Coleoptera: Broscini) for *Broscus aereus* White, 1846. Britton (1949) retained Putzeys' placement of *Oregus aereus* (White) and subsequent addition (Putzeys 1873) of *Mecodema inaequale* Castelnau, 1868 to the genus *Oregus* in his comprehensive review of the New Zealand Broscini. Since 1949 there has been no further taxonomic work on these species, although a brief generic revision of *Oregus* was included by Roig-Junent (2000) during his review of the tribe Broscini. Barbara Barratt (pers. comm. 2001) collected a large series of *O. inaequalis* in the mid-1980s that convinced other entomologists at the time that *O. inaequalis* was a separate species and not in need of synonymising. Continued confusion between *O. inaequalis* and *O. aereus* was evident from the large number of incorrect determinations still present in existing collections. In a brief study of *O. inaequalis*, Jamieson (1999) concluded that there was significant variation in the male genital morphology of *O. aereus* in the Dunedin urban area, sufficient, in her opinion, to indicate the possibility of additional species.

A comprehensive review of the genus was necessary to validate *O. inaequalis* as a separate species, to collate historical records more precisely in order to map the historic distribution (see Pawson & Emberson 2001), and to investigate the possibility of additional species that could be of conservation concern. Our study used a phylogenetic approach based on adult morphological characters (both external and genitalic) and DNA sequencing of partial mitochondrial cytochrome oxidase I (CO I) and NADH dehydrogenase subunit one (ND1) gene regions. These characters were used to infer the species relationships within the genus *Oregus* and to determine if there was support for the presence of additional undescribed species.

## 2. Methods

All known and available specimens from national, and selected international and private collections were examined in the course of the study. A total of 2196 specimens were examined and 153 were dissected for genitalic analysis. Specimens from 17 populations representing the entire geographic range of *Oregus* were chosen as terminal taxa for the morphological analysis (Pawson 2002), which used a total of 27 characters (Pawson 2002) derived from external adult morphology and genitalia. Twenty-two individuals from 12 populations (Fig. 1) were sequenced to produce partial DNA sequences of the CO I and ND1 gene regions (individuals used in the molecular analysis were different from those examined in the morphological analysis, although they were from the same populations). Fitch parsimony (Fitch 1971) was used to analyse the morphological data, and a combination of Fitch parsimony and maximum

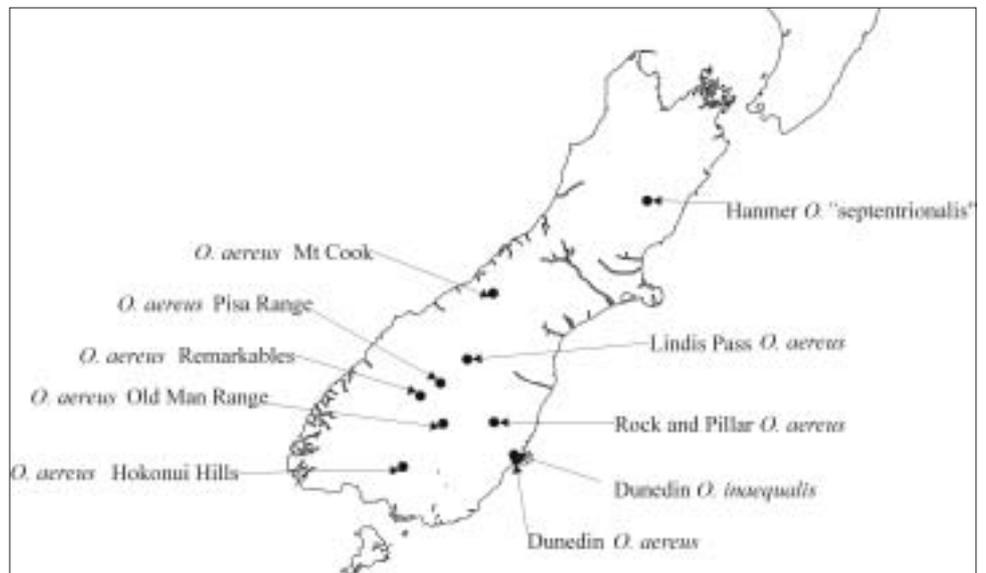


Figure 1. Specimens of *Oregus* collected in South Island, New Zealand, for molecular analysis.

likelihood was used in the analysis of molecular data. *Diglymma clivinooides* was used as an outgroup for both morphological and molecular analyses.

### 3. Results

Morphological analysis based on all 27 characters (Pawson 2002) separated the 17 populations into four distinct groups. The relationships between these groups, however, were inconclusive. The analysis was repeated using a subset of male genitalic characters that proved less homoplasious (Kruskall-Wallis Test  $P < 0.001$ , 1 d.f.) than either external or female genitalic characters. In the second analysis *O. inaequalis* formed a distinct basal clade and the remaining populations were separated into two groups that formed a sister clade to a monophyletic clade of *O. aereus* sensu stricto (Fig. 2).

These two groups from the northern areas of the South Island have been designated new species, *Oregus* “crypticus” and *Oregus* “septentrionalis”. However, these new species are as yet undescribed and the manuscript names used here are unavailable (International Commission on Zoological Nomenclature 1999). These manuscript names will be used in forthcoming formal descriptions.

Molecular analysis of the 12 populations sequenced (Fig. 1) indicate three divisions within the genus *Oregus* (Fig. 3). Unfortunately fresh material of *O.* “crypticus” (necessary for this particular molecular analysis) was unavailable and could not be included in the molecular work. The three divisions identified by the molecular work support those based on male morphological characters.

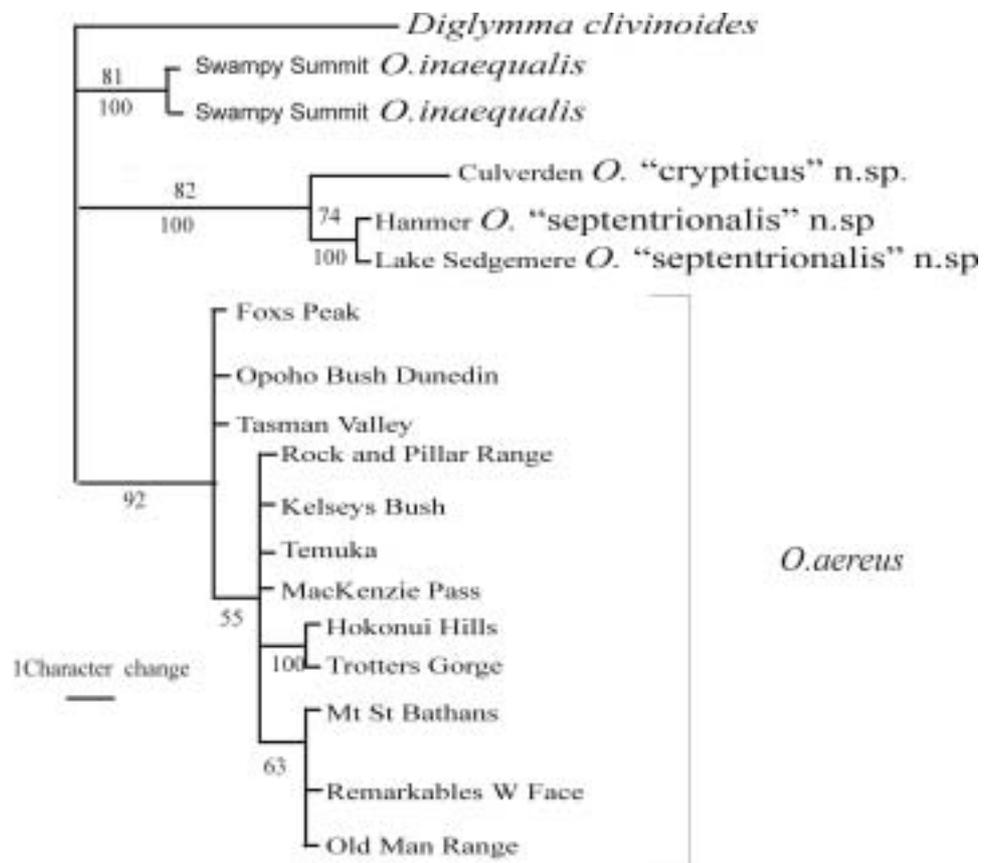


Figure 2. Majority rule consensus phylogram of 38 equally parsimonious trees. Values below the branches represent majority rule (> 50%) consensus values (i.e. a value of 100 means that that particular branch occurred in 100% of the 38 equally parsimonious trees); values above branches indicate bootstrap support from 1000 replicates. Tree length = 23 steps, CI = 0.913, RI = 0.923.

## 4. Discussion

The phylogenetic analysis of morphological and molecular characters indicates that the genus *Oregus* is composed of at least four species, not two as was previously thought. There is no support here for the presence of additional species in the greater Dunedin area as suggested by Jamieson's preliminary morphological investigation (Jamieson 1999). *O. inaequalis* is a distinct species maintaining 2.9–3.05% genetic divergence from *O. aereus* (Pawson 2002) even though they have sympatric distributions to the North of Dunedin city (Fig. 4). *O. aereus* is a widespread species (Fig. 4) with relatively high intraspecific molecular variability (Pawson 2002) compared with the other three species. This greater intraspecific variability is probably attributable to its much wider distribution and lower levels of gene flow between distant populations. Intraspecific variability of even greater extent has been noted in the morphology of New Zealand Broscini in the past (Townsend 1965). However, the level of intraspecific variability in *Oregus* is not sufficient to warrant the subspecies status (external variability but identical male genitalia), as was

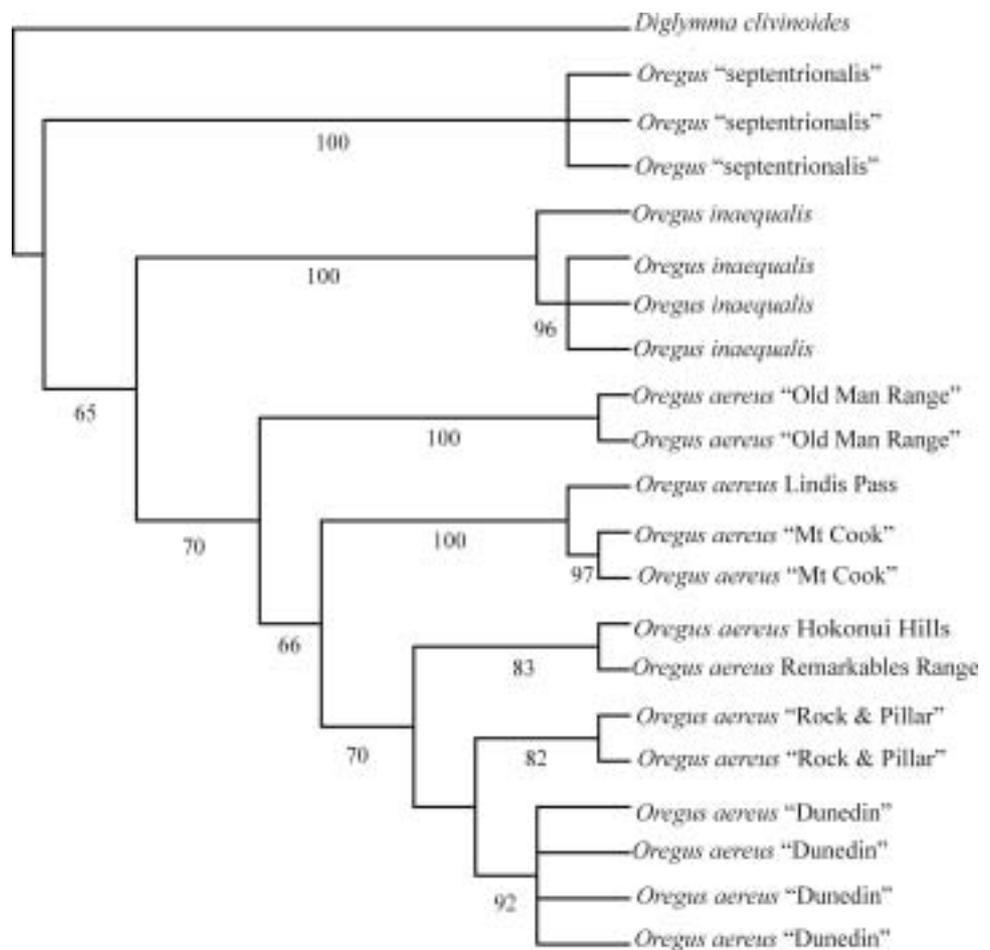


Figure 3. Maximum likelihood tree inferred from the combined COI and ND1 gene regions. Model of sequence evolution TVM + G, ln= 2588.8792; values below the branches indicate bootstrap support from 100 replicates.

attributed to various populations of *Mecodema costellum* Broun 1903 and *Mecodema rugiceps* Sharp 1886 by Townsend (1965). The two new species, *O.* "septentrionalis" and *O.* "crypticus", are distributed in Marlborough and North Canterbury respectively. Initially it was thought that *O.* "crypticus" might have been of conservation concern due to the very limited number of specimens collected. However, Ecki Brockerhoff (Forest Research Institute, unpubl. data) has recently found a very extensive population in Eyrewell Forest.

The placement of *O.* "crypticus" by molecular analysis is unconfirmed, but based on morphology it would appear to be the sister species to *O.* "septentrionalis". Male genitalic characters (specifically the notch in the ventral surface of the male aedeagus) would place *O.* "crypticus" as an intermediate between *O.* "septentrionalis" and *O.* *inaequalis*. Additional material is being sought from the Eyrewell forest population to accurately place *O.* "crypticus" in the molecular phylogeny before the publication of formal descriptions.

Congruence between the different data sets (morphological and molecular) provides greater confidence in the results, i.e. they represent a more accurate

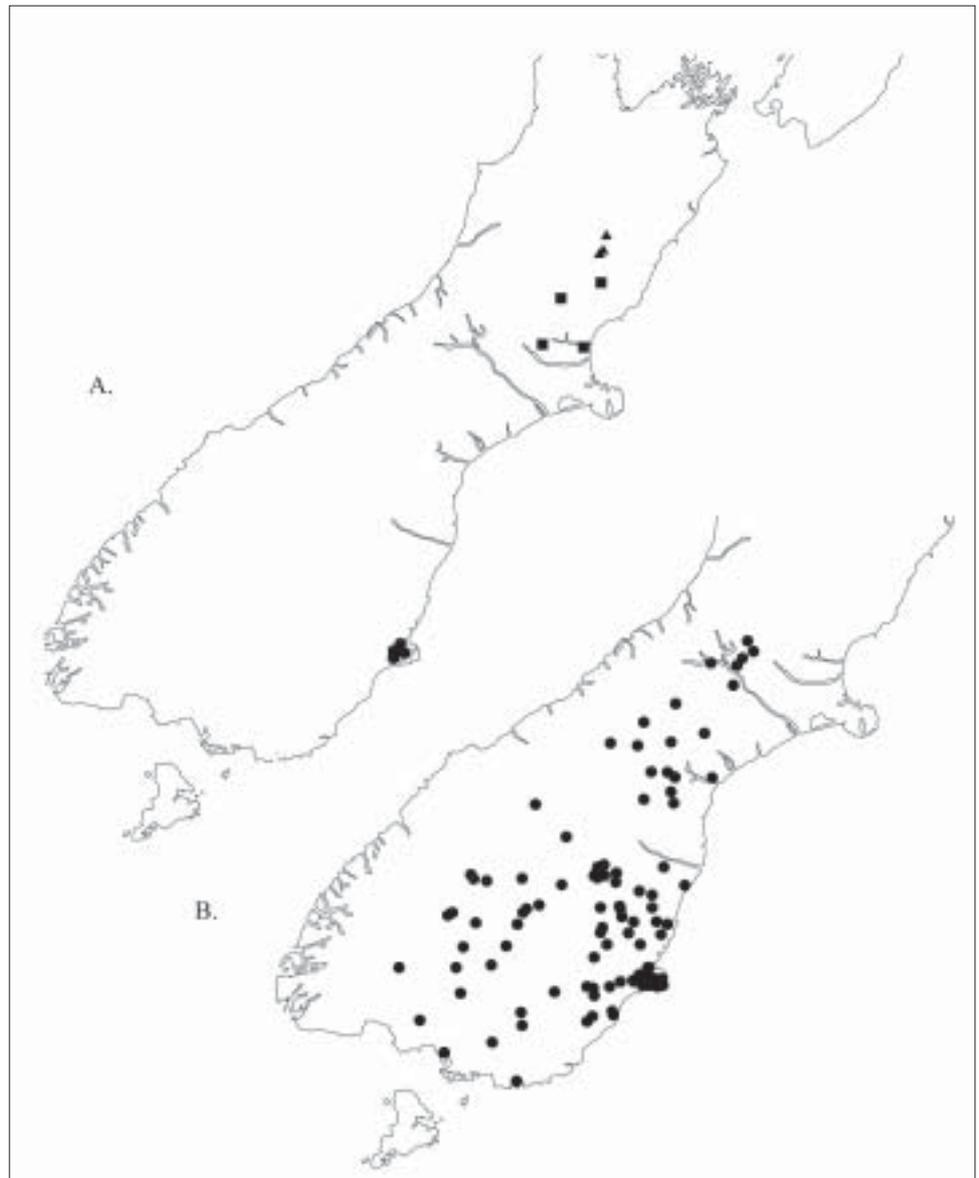


Figure 4. A. Distribution of *Oregus inaequalis* ●, *Oregus* “septentrionalis” ▲, and *Oregus* “crypticus” ■. B. Distribution of *Oregus aereus* ●.

estimate of the true phylogeny (de Queiroz et al. 1995). The words ‘accurate estimate’ are used intentionally, as the true phylogeny can never be known; phylogenetic studies merely attempt to reconstruct the actual relationships between species.

A thorough revision of *Oregus* is in preparation, including a key to species, generic description, redescription of *O. aereus* and *O. inaequalis* and original descriptions and type designations for *O.* “septentrionalis” and *O.* “crypticus”.

## 5. Acknowledgements

The authors would like to acknowledge the assistance of: Bruce McKinlay for providing logistical support and organising funding through the Department of Conservation's Unprogrammed Science Advice Fund; Barbara Barratt for frequent discussions regarding the taxonomy of *Oregus*; Paul Pope from the Dunedin City Council who provided access to Council reserves; Travis Cross, Kathy Hill and Helen Ecroyd for their excellent assistance in the collection of specimens.

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