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Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): a widespread 'mite harvestman' defies DNA taxonomy

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Abstract

Aoraki denticulata **(Arachnida, Opiliones, Cyphophthalmi, Pettalidae), a widespread 'mite harvestman' endemic to the South Island of New Zealand, is found in leaf littler habitats throughout Nelson and Marlborough, and as far south as Arthur's Pass. We investigated the phylogeography and demographic history of *A. denticulata* in the first genetic population-level study within Opiliones. A total of 119 individuals from 17 localities were sequenced for 785 bp of the gene cytochrome *c* oxidase subunit I; 102 of these individuals were from the *Aoraki* subspecies *A. denticulata denticulata* and the remaining 17 were from the subspecies *A. denticulata major*. An extraordinarily high degree of genetic diversity was discovered in *A. denticulata denticulata*, with average uncorrected *p*-distances between populations as high as 19.2%. AMOVA, average numbers of pairwise differences, and pairwise F_{ST} values demonstrated a significant amount of genetic diversity both within and between populations of this subspecies. Phylogenetic analysis of the data set revealed many well-supported groups within *A. denticulata denticulata*, generally corresponding to clusters of specimens from single populations with short internal branches, but separated by long branches from individuals from other populations. No haplotypes were shared between populations of the widespread small subspecies, *A. denticulata denticulata*. These results indicate a subspecies within which very little genetic exchange occurs between populations, a result consistent with the idea that Cyphophthalmi are poor dispersers. The highly structured populations and deep genetic divergences observed in *A. denticulata denticulata* may indicate the presence of cryptic species. However, we find a highly conserved morphology across sampling localities and large genetic divergences within populations from certain localities, equivalent to those typically found between populations from different localities. Past geological events may have contributed to the deep genetic divergences observed between sampling localities; additionally, the high divergence within populations of *A. denticulata denticulata* suggests that the rate of COI evolution may be accelerated in this taxon. In contrast, the larger subspecies *A. denticulata major* shows much less differentiation between and within sampling localities, suggesting that it may disperse more easily than its smaller counterpart. The fact that the remarkable genetic divergences within populations of *A. denticulata denticulata* from certain localities are equivalent to divergences between localities poses a challenge to the rapidly spreading practice of DNA taxonomy**

Keywords: barcoding, cryptic species, mitochondrial DNA, Opiliones, phylogeography, taxonomy

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Introduction

New Zealand has a turbulent geological history, including rifting from Gondwana *c*. 80 million years ago (Ma), near total submersion of land during the Oligocene marine transgression episode that resulted in a severe biological bottleneck *c*. 35 Ma, the dramatic uplift of the Southern Alps 5–2 Ma, and substantial Pleistocene glaciation (Hayes & Ringis 1973; Stevens 1980; Batt *et al*. 2000; Chamberlain & Poage 2000). Due to its Gondwanan origin and subsequent considerable isolation from other major landmasses, the fauna of New Zealand has characteristics typical of both continents and islands (Cooper & Millener 1993; Daugherty *et al*. 1993; Waters & Craw 2006; Trewick 2007). Vicariance, dispersal, disjunction, and radiations have all played roles in shaping the diversity of life seen there today, and the high rates of endemism found in New Zealand's threatened habitats have led to its designation as one of the world's biodiversity hotspots (Myers *et al*. 2000). New Zealand in general, and the South Island in particular, have been the subject of several phylogeographical studies which link the geological history of the archipelago to patterns of genetic differentiation within various endemic species (e.g. Trewick *et al*. 2000; Buckley *et al*. 2001; Trewick & Wallis 2001; Trewick 2001; Arensburger *et al*. 2004; Trewick & Morgan-Richards 2005).

In this study, we undertake a phylogeographical analysis of a leaf-litter arachnid from the suborder Cyphophthalmi, a group which has a remarkably high diversity in New Zealand (Forster 1948, 1952; Boyer & Giribet 2003, 2007). Cyphophthalmi is a small suborder of Opiliones — one of the 11 orders of arachnids, commonly known as daddy long-legs or harvestmen. These tiny cryptic arachnids range in length from approximately 1–5 mm and are typically found in leaf litter habitats all over the world. The cyphophthalmid family Pettalidae is unequivocally monophyletic and has a classic Southern Gondwanan distribution with members in Australia, Chile, Madagascar, New Zealand, South Africa and Sri Lanka, a pattern which is inferred to be the result of vicariance (Boyer & Giribet 2007). Thanks to local arachnologist Ray Forster, the cyphophthalmid fauna of New Zealand is perhaps the best known in the world, with 29 described endemic species and subspecies, most described by Forster himself (Forster 1948, 1952). The South Island is home to 21 known species and subspecies currently classified in three genera (Boyer & Giribet 2007): *Rakaia* Hirst, 1925, with 11 described and two undescribed species and subspecies ranging throughout the east and south coasts from the Marlborough Sounds to Stewart Island; *Aoraki* Boyer & Giribet, 2007, with six described and one undescribed species and subspecies; and the monotypic genus *Neopurcellia* Forster, 1948, ranging along the west coast from Lake Kaniere to Te Anau.

Cyphophthalmi are notoriously poor dispersers, with most species recorded from a handful of localities within a very small range (Juberthie & Massoud 1976; Shear 1980; Giribet 2000; Boyer & Giribet 2003; Boyer *et al*. 2005), and a single known potential example of transoceanic dispersal known in the suborder (Clouse & Giribet 2007). As a general rule, cyphophthalmid species that inhabit proximal ranges are closely related to one another evolutionarily, and the likely mechanism of diversification within this group is allopatric speciation when geographical barriers arise (Boyer & Giribet 2007; Boyer *et al*. 2007). To date, little is known about the amount of genetic diversity or gene flow within cyphophthalmid species, although results from higher-level phylogenetic studies indicate strikingly high genetic diversity in the mitochondrial gene cytochrome *c* oxidase subunit I (Boyer *et al*. 2005; Schwendinger & Giribet 2005), the most commonly used population-level marker in animal studies.

This study represents the first population genetic analysis from any species of Opiliones. Our subject is *Aoraki denticulata* (Forster, 1948), one of the world's most widespread cyphophthalmid species. *A. denticulata* lives in forest leaf litter habitats throughout Marlborough, Nelson, and south to Arthur's Pass, an area that includes more continuous native *Nothofagus* beech forest than any other region of New Zealand (Smith 2005). The species was described originally as two subspecies: *Rakaia denticulata denticulata* Forster, 1948, based on type material from Starvation Ridge in what is now Kahurangi National Park, and *Rakaia denticulata major* Forster (1948) from Arthur's Pass. The two subspecies differ only in size: 'a characteristic noted during the study of New Zealand cyphophthalmids is the extremely slight variation in size shown within a species and hence I have no hesitation in naming [*Rakaia denticulata major*] as a subspecies' (Forster 1948: 103). Both have since been transferred to the genus *Aoraki*, with *A. denticulata denticulata* designated as the type species of the genus (Boyer & Giribet 2007). *A. denticulata denticulata* is now known from many localities, but *A. denticulata major* is still restricted to collecting sites near Arthur's Pass, encompassing a mere 35-km stretch of the Pass from Otira to Cass (Fig. 1). Some of the distinguishing characters of *A. denticulata* include the presence of two scopulae originating from the posterior ventral surface of the anal plate (Fig. 2) and a fourth tarsus of the male with a distinctively rectangular shape (compared with other Cyphophthalmi), in addition to the particular ornamentation of the cuticular surface, and the round protuberance at the base of the adenostyle (Fig. 3) — these characters are easily diagnosable and are not found in this combination in any other species.

Significant genetic differentiation within *A. denticulata* and a strong geographical trend within the data are expected because of the inferred poor dispersal ability of Cyphophthalmi.

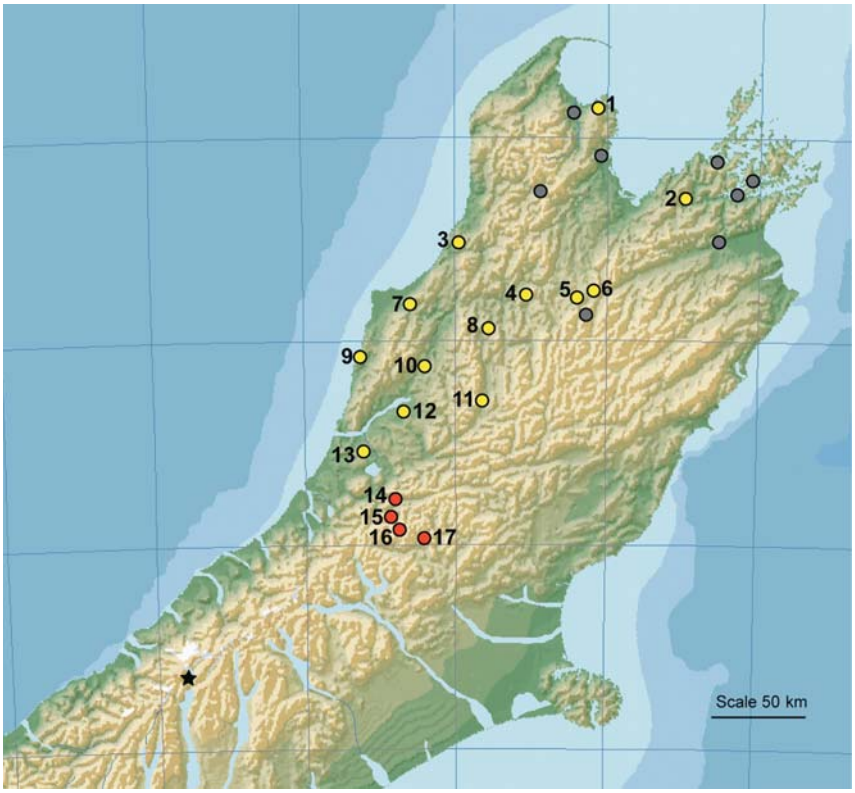

Fig. 1 Map of northern South Island, New Zealand, showing *Aoraki denticulata* localities. Yellow circles indicate *A. denticulata denticulata* localities included in this study, grey circles indicate known *A. denticulata denticulata* localities not included in this study, red circles indicate *Aoraki denticulata major* localities, and the star indicates the locality for *Aoraki longitarsa*.

The range inhabited by this species includes areas that were strongly modified by Pliocene orogeny and subsequent Pleistocene glaciation, during which time areas of grassland, shrubland, and scattered forest surrounded the ice and alpine vegetation of the Southern Alps (McGlone *et al*. 1993). These past events are expected to affect the present-day population structure of *A. denticulata*. In addition to investigating patterns of population subdivision and demographic history, we test the taxonomic hypothesis of reciprocally monophyletic subspecies *A. denticulata denticulata* and *A. denticulata major.*

Over the past few years, DNA taxonomy and barcoding have emerged as major aims of many zoologists working with molecular data (e.g. Hebert *et al*. 2003; Tautz *et al*. 2003). For example, the Consortium for the Barcode of Life (CBOL) has been established as 'an international initiative devoted to developing DNA barcoding as a global standard in taxonomy' (see http://www.barcoding.si.edu/). CBOL describes DNA barcoding as 'a technique that uses a short gene sequence from a standardized region of the genome as a diagnostic "biomarker" for species. Different species have different DNA barcodes, making it possible to use barcodes to identify specimens (and) discover possible new species, and to make taxonomy more effective for science and society'. Undoubtedly, DNA sequences have been a valuable tool for systematists and taxonomists for at least two decades, many of whom have used DNA to guide or accompany taxonomic descriptions (e.g. Brower 1996; Edgecombe & Giribet 2003; Carranza & Amat 2005) or to allow identification of species of commercial interest for conservation purposes (e.g. DeSalle & Birstein 1996; Palumbi & Cipriano 1998; Baker *et al*. 2000). The novel aspect of the new barcoding approach (DNA taxonomy *sensu* Tautz *et al*. 2003) is the proposed standardization of the use of sequence data in taxonomic practice (Rubinoff & Holland 2005).

Many have argued against DNA taxonomy on several bases, including concern about discarding the information content of morphology, the utility of both morphology and ecology for applications such as conservation, and concern for its impact on taxonomic practices in developing countries (Lipscomb *et al*. 2003; Seberg *et al*. 2003; Rubinoff & Sperling 2004). DNA barcoding (as opposed to DNA taxonomy) has found many utilities in taxonomy and conservation sciences. Still, there are important concerns, such as whether a 'one gene fits all' concept is appropriate across all of plant and animal life (Rubinoff & Holland 2005), and how to use or read so-called barcodes once they are generated (DeSalle *et al*. 2005; Meier *et al*. 2006). In addition, several studies have shown the failure of the barcoding approach for identification purposes, the main objective of the barcoding initiative (e.g. Will & Rubinoff 2004; Meier *et al*. 2006), precisely because of the phenomenon of intraspecific variability. Here we discuss the barcoding approach in light of the novel data generated for *A. denticulata*.

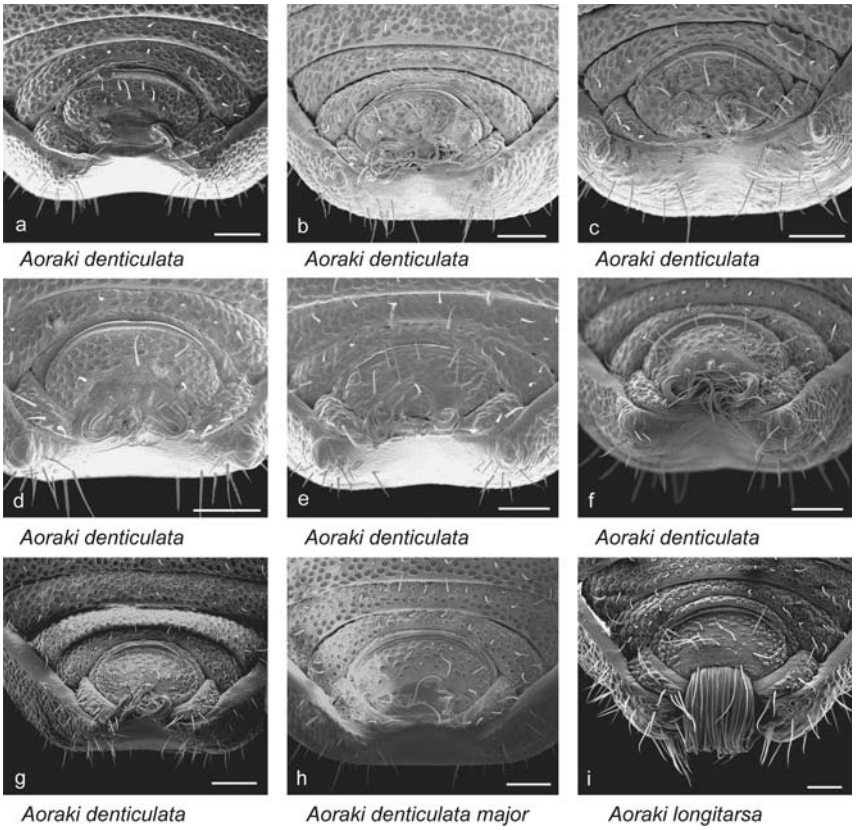

Fig. 2 Scanning electron micrographs of the posterior ventral region of male specimens from different populations. Scale bar 100 μm a. *Aoraki denticulata denticulata*, locality 5, deposited at MCZ under accession number DNA100961 (individual 5.1); b. *A. denticulata denticulata*, locality 4, deposited at MCZ under accession number DNA100941 (4.1); c. *A. denticulata denticulata*, locality 3, deposited at MCZ under accession number DNA100948 (3.1); d. *A. denticulata denticulata*, locality 9, deposited at MCZ under accession number DNA100955 (9.1); e. *A. denticulata denticulata*, locality 10, deposited at MCZ under accession number DNA100964 (10.1); f. *A. denticulata denticulata*, specimen collected by R. Forster 25.IV. 1977 from Lewis Pass, deposited Otago Museum; g. *A. denticulata denticulata*, locality 2, deposited at MCZ under accession number DNA101811 (2.1); h. *A. denticulata major*, locality 17, deposited at MCZ under accession number DNA100959 (17.1); i. *Aoraki longitarsa* MCZ DNA101806.

Methods

Collection and identification

Specimens were collected from leaf litter either by sifting *in situ*, sorting, and direct collection, or with a Winkler apparatus — a canvas funnel where mesh bags containing soil and leaf litter hang to allow soil arthropods to walk about and fall into a collecting vial. Collecting expeditions occurred in 2003, 2004, and 2006. Appendix I lists all localities included in this study and detailed collecting information. GPS data were taken at each collecting site, and these coordinates were used as input to ArcGIS (ESRI) (see Appendix I) to generate a precise locality map (Fig. 1).

Animals were identified and compared to holotypes using a Leica MZ 12.5 stereomicroscope. Males of *Aoraki denticulata denticulata* from six localities, along with a male of *A. denticulata major* and the closely related species *Aoraki longitarsa* (Forster, 1952), were mounted for scanning electron microscopy (SEM) to perform morphological comparisons focusing on all known characters of systematic importance in Cyphophthalmi (e.g. Giribet & Boyer 2002; Boyer & Giribet 2007). SEM was performed using an FEI Quanta 200 microscope. The resulting digital scanning electron micrographs were used to further confirm species identity (Figs 2 and 3).

DNA extraction and sequencing

Genomic DNA was extracted from a single leg from each individual as described in Boyer *et al*. (2005) using QIAGEN's DNeasy extraction kit. DNA was also extracted from the specimens employed for SEM study. A 785 bp region of cytochrome *c* oxidase subunit I (COI hereafter) was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAG ATATTGG-3') (Folmer *et al*. 1994) and HCOoutout (5'-GTAAATATATGRTGDDGCTC-3') (Schwendinger & Giribet 2005). The double-stranded polymerase chain reaction (PCR) products were visualized by 1% agarose gel electrophoresis and purified using QIAGEN QIAQuick spin columns. The purified PCR products were sequenced directly using ABI Big Dye Terminator version 3.0 (Applied Biosystems) following standard protocols described by the manufacturer. The BigDye-labelled PCR products were cleaned with AGTC Gel Filtration Cartridges or Plates (Edge BioSystems). The sequence reaction products were then analysed using an ABI PRISM 3730 Genetic Analyzer. Sequences were edited in SEQUENCHER 4.5 (Gene Codes) and aligned in MACGDE: genetic data environment for MacOS X, version 2.2 (Linton 2005). Alignments were trivial, as sequences did not vary in length between individuals.

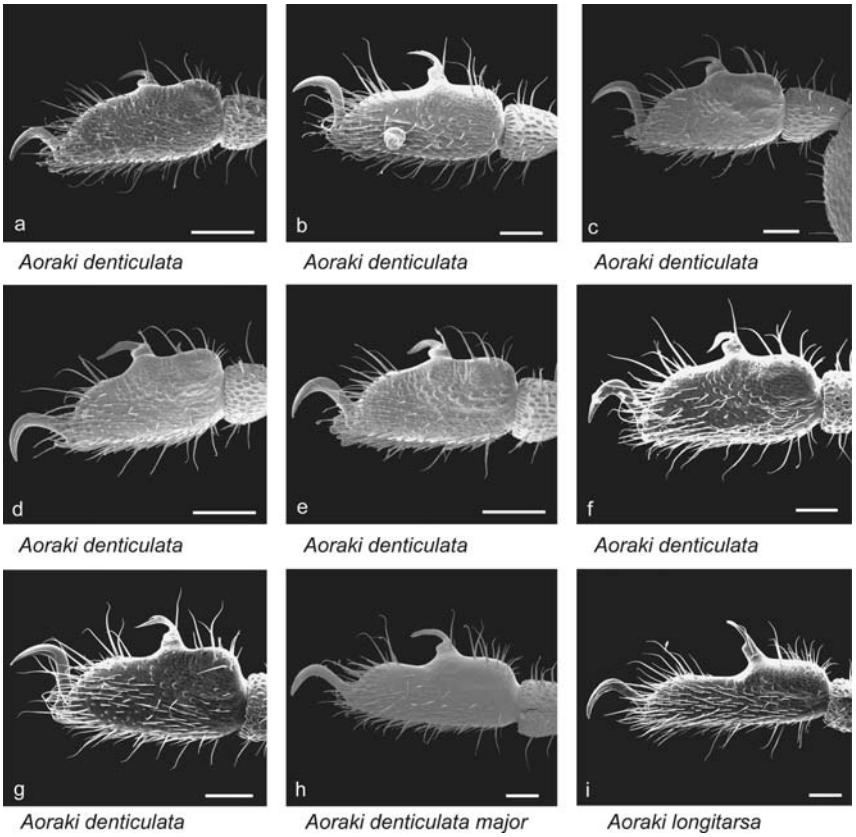

Fig. 3 Scanning electron micrographs of male tarsus IV of different specimens. Scale bar 100 μm. a. *Aoraki denticulata denticulata*, locality 5, accession number MCZ DNA100961 (individual 5.1); b. *A. denticulata denticulata*, locality 4, accession number MCZ DNA100941 (4.1); c. *A. denticulata denticulata*, locality 3, accession number MCZ DNA100948 (3.1); d. *A. denticulata denticulata*, locality 9, accession number MCZ DNA100955 (9.1); e. *A. denticulata denticulata,* locality 10, accession number MCZ DNA100964 (10.1); f. *A. d. denticulata*, specimen collected by R. Forster 25.IV. 1977 from Lewis Pass, deposited Otago Museum; g. *A. denticulata denticulata,* locality 2, accession number MCZ DNA101811 (2.1); h. *A. d. major,* locality 17, accession number MCZ DNA100959 (17.1); i. *Aoraki longitarsa* MCZ DNA101806.

Population genetic and phylogeographic analyses

Population genetic analyses were performed using ARLEQUIN version 3.01 (Excoffier *et al*. 2005) and DNASP version 4.10.8 (Rozas *et al*. 2003, 2006). Standard diversity indices, including number of haplotypes (*Nh*), haplotypic diversity (*h*), nucleotide diversity (π_n) and average number of pairwise differences were calculated to assess diversity within each population. To evaluate differentiation between populations, we calculated the average number of pairwise differences between populations, as well as population pairwise F_{ST} values (based on the observed number of pairwise differences) whose significances were assessed through 10 000 permutation tests. An analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) was performed to test hierarchical models of genetic variance using pairwise differences as a measure of divergence (16 000 permutations). The data were partitioned into geographical locations, and two different AMOVA tests were run: one with populations grouped by subspecies (*A. denticulata denticulata* vs. *A. denticulata major*), and one without taxonomic groups. Localities with one or two individuals were eliminated from pairwise population comparisons and AMOVA tests.

To test the hypothesis of postglacial population expansion in *A. denticulata* Tajima's *D* (Tajima 1989) and Fu's F_S (Fu 1997) were calculated for all *A. denticulata* specimens considered as one group. Although Tajima's *D* was originally developed to test for selection, a negative value suggests the presence of an excess of low-frequency haplotypes, which would be expected under an expansion scenario (Aris-Brosous & Excoffier 1996). As an additional test of population expansion, Ramos-Onsins and Rozas's R_2 test (Ramos-Onsins & Rozas 2002), was performed for the entire data set using DNASP version 4.10.8 (Rozas *et al*. 2003, 2006).

A haplotype network was estimated with TCS version 1.18 (Clement *et al*. 2000), which implements the statistical parsimony procedure (Templeton *et al*. 1992; Crandall 1994). This method estimates an unrooted tree and provides a 95% plausible set for the relationships among haplotypes.

Phylogenetic analyses

A phylogenetic approach to this data set was undertaken using maximum likelihood and parsimony as optimality criteria. For these analyses, a number of outgroup taxa were used from all three of the Cyphophthalmi genera present in New Zealand, including representatives of the following species: *Aoraki calcarobtusa westlandica* (Forster, 1952), *A. healyi* (Forster, 1948), *A. longitarsa* (Forster, 1952), *A. tumidata* (Forster, 1948), a putative new species of *Aoraki* from Mount Stokes, *Neopurcellia salmoni* (Forster, 1948), *Rakaia*

Table 1 Diversity measures for populations of *Aoraki denticulata* included in this study, including locality numbers (corresponding to those in Fig. 1), sample size (*n*), number of haplotypes (*Nh*), number of polymorphic sites (*Np*), haplotype diversity (*h*) and nucleotide diversity (π_n)

Population	Locality	*Aoraki* ssp.	*n*	*Nh*	*Np*	*h*	π_n
Totaranui	1	*denticulata*	1	1	NA	NA	NA
Pelorus Bridge	2	*denticulata*	9	3	2	0.5556 ± 0.1653	0.000778 ± 0.000766
Karamea Bight	3	*denticulata*	9	9	214	1.0000 ± 0.0524	0.141295 ± 0.076194
Longford	4	*denticulata*	17	11	74	0.9044 ± 0.0572	0.021300 ± 0.011167
Lake Rotoiti	5	*denticulata*	5	4	11	0.9000 ± 0.1610	0.006369 ± 0.004351
St. Arnaud	6	*denticulata*	4	4	7	1.0000 ± 0.1768	0.004459 ± 0.003409
10 Mile Creek	7	*denticulata*	8	8	48	1.0000 ± 0.0625	0.016924 ± 0.009721
Burnbrae	8	*denticulata*	2	2	129	1.0000 ± 0.5000	0.164331 ± 0.164967
Punakaiki	9	*denticulata*	18	10	93	0.8431 ± 0.0771	0.015936 ± 0.008437
Reefton	10	*denticulata*	9	6	119	0.8333 ± 0.1265	0.058528 ± 0.031848
Rahu Scenic Reserve	11	*denticulata*	12	8	198	0.8939 ± 0.0777	0.117970 ± 0.061518
Ahaura	12	*denticulata*	4	4	6	1.0000 ± 0.1768	0.004246 ± 0.003269
Mitchell	13	*denticulata*	9	7	9	0.9444 ± 0.0702	0.003185 ± 0.002145
Otira	14	*major*	1	1	NA	NA	NA
Bealy Gorge Track	15	*major*	1	1	NA	NA	NA
Greyney's Shelter	16	*major*	9	2	2	0.4167 ± 0.1907	0.000566 ± 0.000627
Cass	17	*major*	6	2	1	0.3333 ± 0.2152	0.000425 ± 0.000559

antipodiana (Hirst, 1925), *R. dorothea* (Phillipps & Grimmett, 1932), *R. lindsayi* (Forster, 1952), *R. magna australis* (Forster, 1952), *R. minutissima* (Forster, 1948), *R. solitaria* (Forster, 1948), *R. stewartiensis* (Forster, 1948), and four putative new species of *Rakaia* from the Akatarawa Divide, Hineway Reserve, Kapiti Island, and Wi Toko Scenic Reserve. The model of sequence evolution that best fit the data was selected using MODELTEST 3.7 (Posada & Crandall 1998) according to the Akaike information criterion (AIC; Posada & Buckely 2004). We analysed the data under the chosen model using maximum likelihood in PAUP* portable version 4.0b10 for Unix (Swofford 2002) on the Harvard University Center for Genomic Research supercomputing cluster (portal.cgr.harvar.edu), using a heuristic approach with 100 random addition replicates and tree-bisection–reconnection (TBR) branch swapping. We ran 100 bootstrap replicates (due to computing limitations) under the same search strategy (100 random addition replicates and TBR branch swapping for each bootstrap replicate) to assess support for the clades recovered. In addition, we analysed the data using parsimony as an optimality criterion also following a heuristic approach with 1000 random addition replicates with equal weighting of transitions and transversions, and again, assessed support with 1000 bootstrap replicates.

Results

A total of 119 individuals of *Aoraki denticulata* from 17 localities were sequenced, including 17 specimens from the large subspecies *A. dnticulata major* (Table 1, Appendix I). From the 119 sequences, 84 haplotypes were identified with a total of 169 variable sites (21.5% of 785 bp total length) (Appendix II). Three localities (1, 14, and 15; see Fig. 1) yielded a single successfully sequenced individual and one locality (8) yielded two successfully sequenced individuals; these individuals were included in the phylogenetic analysis of the data set but not in population genetic analyses. Nucleotide variation was scattered across the entire sequenced region, with 252 synonymous substitutions and 43 nonsynonymous substitutions.

Population genetics and demographic statistics

Nucleotide diversity (π_n) and haplotype diversity (*h*) within populations are summarized in Table 1 (see also Appendix II). No haplotypes were shared between different localities, except for the Arthur's Pass *A. denticulata major* populations, in which haplotype H81 was found at three of four localities. Four localities had *h*-values of 1.00, meaning that no haplotypes were shared among the population's members (Appendix II).

F_{ST} values of population pairs (Table 2) were greater than 0.8 in most cases, indicating that genetic variation is much larger between locations than within locations, showing strong geographical structure within the species. All F_{ST} comparisons were significant ($P < 0.05$) with a single exception — the comparison between the *A. denticulata major* populations from localities 16 and 17.

The average number of uncorrected pairwise differences among sequences from *A. denticulata* when all data were considered was 113.0 (14.4%). Average pairwise differences

Table 2 Pairwise F_{ST} values among locations. Comparisons that were significant at the $P < 0.05$ level are indicated by asterisks. Populations represented by one or two sequenced individuals were excluded (localities 1, 8, 14, 15)

	2	3	4	5	6	7	8	9	10	11	12	13	16	17
2	0													
3	0.587**	0												
4	0.894**	0.570**	0											
5	0.981**	0.492*	0.873**	0										
6	0.987**	0.422*	0.396*	0.962*	0									
7	0.945**	0.552*	0.875**	0.915*	0.918*	0								
8	0.851*	0.236	0.782*	0.737*	0.712	0.744*	0							
9	0.931**	0.644**	0.886**	0.916**	0.915*	0.898*	0.825*	0						
10	0.791**	0.418*	0.761**	0.735*	0.720*	0.739**	0.379*	0.792**	0					
11	0.589**	0.314**	0.623**	0.540**	0.510*	0.529*	0.04	0.638**	0.416**	0				
12	0.988**	0.466*	0.881*	0.964*	0.972*	0.906*	0.613*	0.898*	0.552*	0.466*	0			
13	0.986**	0.608*	0.906**	0.973*	0.978*	0.930*	0.680*	0.926**	0.780**	0.313**	0.976*	0		
16	0.994**	0.608**	0.911**	0.982**	0.990**	0.947**	0.859*	0.936**	0.816**	0.614**	0.990**	0.988**	0	
17	0.994**	0.549**	0.900**	0.977**	0.988**	0.936**	0.799*	0.929**	0.781**	0.564**	0.988**	0.986**	–0.008	0

within populations ranged from 0.3 to 129.0 (0 to 16.4%), and average pairwise differences between populations ranged from 0.4 to 151.0 (0 to 19.2%) when the *A. denticulata major* populations were considered, or 70.2 to 151.0 (8.9 to 19.2%) when the *A. denticulata major* populations were not considered. (Table 3).

The AMOVA (Table 4) revealed that the majority of variation occurs between rather than within populations, despite very high *h*-values within most populations. When populations were not grouped by subspecies, 77.22% of the variation was found between populations, and 22.78% within populations. When populations were grouped by subspecies, 27.15% of the variation occurred between subspecies, 54.76% between populations within each subspecies, and 18.09% within populations. All fixation indices were high and significant at $P < 0.05$, suggesting that a significant amount of differentiation is observed at all hierarchical levels (Table 4).

When all *A. denticulata* specimens were considered in one group, Tajima's *D* (0.21396), Fu's *F*s (–0.295), and the R_2 value (0.1542) were all nonsignificant ($P > 0.10$).

Phylogeography

The haplotype network generated by TCS under a 95% connection limit consisted of 14 separate unlinked networks plus 10 haplotypes that did not group with any others. The networks generated by TCS each contained only haplotypes from a single locality, with two exceptions: a network including all *A. denticulata major* localities, and a network which linked haplotypes from localities 4 and 6, although separated by 11 mutational steps. Therefore, we did not perform a nested clade analysis with this data set.

The phylogenetic tree constructed from all haplotypes and selected outgroups (39 individuals from other New Zealand Cyphophthalmi) under maximum likelihood (–Log L = 13668.03717) indicates that *A. denticulata major* consistently formed a clade nested within *A. denticulata denticulata* (Fig. 4). *A. denticulata major* is the sister clade to the group of individuals from locality 2, Pelorus Bridge in Marlborough. Several other clades were retrieved within the *A. denticulata* assemblage: a west-central clade containing populations from 3 and 7 to 13; a northwest clade containing populations 3, 4, and 6; and a northern clade containing populations 1 and 5. Every clade from localities 3, 10, and 11, each of which appeared in two separate parts of the tree, included at least one male. All males from these localities were unequivocally identified as *A. denticulata denticulata* using stereomicroscopy and, for a subset of individuals, SEM (see Figs 2 and 3). One surprising result of this study is that *Aoraki longitarsa*, a species known only from one locality in Aoraki/Mount Cook National Park (five-point star in Fig. 1), nested within *A. denticulata.* This result was found in the maximum likelihood analysis as well as in the strict consensus of all shortest trees from the parsimony analysis (850 equally parsimonious trees of 2968 steps; consistency index of 0.254; retention index of 0.841). However, this result received less than 50% bootstrap support in the maximum likelihood analysis and 58% bootstrap support in the parsimony analysis (Fig. 4). This placement of *A. longitarsa* within *A. denticulata* has also been found in other analyses including more markers (see discussion below).

Discussion

The male specimens identified as *Aoraki denticulata denticulata* and *Aoraki denticulata major* are easily identified using a stereomicroscope, and identification of males from all the localities has been confirmed with the use of SEM

Table 3 Average pairwise differences among and within populations. Populations represented by single sequenced individuals were excluded (localities 1, 14, 15)

	2	3	4	5	6	7	8	9	10	11	12	13	16	17
2	**0.61**													
3	135.04	**110.92**												
4	105.16	124.95	**16.72**											
5	110.31	136.31	110.35	**5**										
6	109.11	124.83	21.01	113.25	**3.5**									
7	119.35	143.43	124.85	120.35	125.25	**13.29**								
8	122	151	130.32	128.1	132.88	127.25	**129**							
9	124.19	138.32	127.65	130.02	128.89	124.78	132.97	**12.51**						
10	111.11	134.7	114.99	117.2	116.25	116.86	103.17	116.69	**45.94**					
11	125.31	147.44	132.49	135.63	132.65	124.04	106.58	129.04	121.88	**92.61**				
12	117.5	136.5	119.53	120.25	122	108.63	101.63	107.11	70.17	120.21	**3.33**			
13	108.89	144.78	125.92	121.73	128.58	107.96	73.61	124.65	110.26	73.81	112.64	**2.5**		
16	88.22	142.12	124.93	106.62	124.11	122.72	126.67	133.95	126.31	133.31	124.72	118.33	**0.44**	
17	88.17	142.06	124.82	106.57	124	122.67	126.33	133.83	126.15	133	124.33	117.94	0.39	**0.33**

Below diagonal: average number of pairwise differences between populations (PiXY).
Diagonal elements (bold): average number of pairwise differences within population (PiX).

(see Figs 2 and 3). Taxonomy within the genus *Aoraki* relies heavily on the tarsus of the male leg IV and on the anal region of males, and all the specimens studied are consistent with the taxonomic definition of the species. In all other cases of sympatry of Cyphophthalmi species, both in New Zealand and elsewhere, morphological differentiation is clearly evident in adult males. To confirm that the genetic variation we found is not reflected in the morphology of our study organism, we went back and checked the male specimens of *A. denticulata denticulata* and *A. denticulata major* from each population. We paid special attention to populations that appear in two clades (e.g. population 10) and in each case found that the individuals from the two clades were identical for all characters.

Pairwise population comparisons employed in this study indicated that there is little genetic exchange between populations of the widespread subspecies *A. denticulata denticulata*, even when they are separated from each other by as little as 10 km. Additionally, no haplotypes were shared between *A. denticulata denticulata* localities, although certain populations (i.e. 3, 10, 11) contain haplotypes from different genetic lineages, suggesting that rare long-distance dispersal events may occur. F_{ST} values were extremely high across the subspecies *A. denticulata denticulata*, and AMOVA fixation indices between geographical localities were highly significant. In addition, the TCS analysis failed to link populations to each other with greater than 95% probability. All of these results are consistent with previous ideas about high rates of endemism and narrow species ranges for Cyphophthalmi, and suggest that in general, these animals do not travel far during the course of their lives. This general trend of lack of shared haplotypes between populations, which is not unexpected in animals with poor dispersal capabilities, was accompanied by a surprisingly high haplotypic diversity within populations, with many populations consisting of collections of unique haplotypes not shared by any other individuals.

A different trend was seen in the subspecies *A. denticulata major*, as one haplotype was shared among three of the four Arthur's Pass localities where the subspecies lives (Appendix II). These three localities are particularly close together, all occurring within a 20-km stretch of the Pass (Fig. 1: localities 15–17). Intra- and inter population differences were lower in *A. denticulata major*, suggesting that this subspecies may be more capable of dispersal, perhaps due to its larger size when compared to *A. denticulata denticulata.*

In all the other species of Cyphophthalmi inhabiting New Zealand, geographically proximate (and even sympatric) populations of genetically independent lineages show clear morphological differentiation that is easily diagnosable in male individuals. It is possible that *A. denticulata denticulata* represents a unique case in which speciation has occurred in the absence of morphological divergence, as has been reported in other groups of arachnids (e.g. Bond *et al*. 2001). However, this scenario requires positing a different cryptic species at almost every locality sampled in this study, and two sympatric cryptic species at locality 3, another pair at locality 10, and yet another at locality 11. Moreover, our phylogeny shows that branches within *A. denticulata* are generally longer than branches between other pettalid species in the genera *Aoraki* and *Rakaia* — suggesting a faster rate of molecular evolution in our study taxon than in closely related species. The high *h* number within sampling localities coupled with the enormous genetic distances among populations is also consistent with an increased rate of COI evolution in this taxon, a scenario that is also consistent with the high degree of intrapopulation divergence and the lack of resolution of

Table 4 AMOVA results. Each population was considered as a separate group, except for the *Aoraki denticulata major* populations from the four Arthur's Pass localities, which were considered in one group. Asterisks indicate significance at the $P < 0.05$ level. Populations represented by one or two sequenced individuals were excluded (localities 1, 8, 14, 15)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of total variation	Fixation indices
AMOVA grouping by subspecies					
Among groups	1	899.648	20.64758 Va*	27.15	F_{CT}: 0.27149*
Among populations within groups	11	4300.763	41.64286 Vb*	54.76	F_{SC}: 0.75162*
Within populations	106	1458.681	13.76115 Vc*	18.09	F_{ST}: 0.81906*
Total	118	6659.092	76.05158		
AMOVA grouping by population					
Among populations	12	5200.411	46.65924 Va*	77.22	F_{ST}: 0.77224*
Within populations	106	1458.681	13.76115 Vb	22.78	
Total	118	6659.092	60.42039		

d.f., degree of freedom.
Significance tests using 16 000 permutations.

most basal nodes in the phylogeny. Another possibility explaining the current high genetic differentiation in *A. denticulata denticulata* is a genetic admixture of haplotypes following incipient differentiation as a result of habitat fragmentation, for example during Pliocene orogeny or Pleistocene glaciation, and before morphological differentiation was achieved. If this were the case, genetic distances between incipient species within the *A. denticulata denticulata* complex remain larger than distances between morphologically well-defined species of *Aoraki* (Fig. 4).

Geological context

New Zealand has a very complex and eventful geological history. During the Oligocene drowning event, the land area of the North and South Islands was reduced and then expanded again due to changing sea levels, dwindling to a mere 18% of its present-day size approximately 27 Ma (Stevens 1980; Cooper & Cooper 1995; Trewick & Morgan-Richards 2005). It should be noted that these reconstructions remain controversial and some suggest the possibility that during this time New Zealand was submerged entirely (Waters & Craw 2006). Over the last 20–25 million years, the Australian and Pacific tectonic plates have slipped approximately 500 km relative to one another, with the Australian plate moving north. New Zealand featured only limited topographic relief until the Pliocene, when mountain-building began in the South Island (5–2 Ma), during which time all of the major axial mountain ranges were formed. The development of these mountains provided conditions for the formation of extensive glaciers, which reached their maximum during the Pleistocene approximately 20 000 years ago (Stevens 1980; Suggate 1990; Trewick & Wallis 2001; Winkworth *et al*. 2005). Marine inundation, tectonic activity, orogeny, and glaciation are all phenomena that contributed to fragmentation of the forests of the South Island during the last 30 million years.

During the last glacial maximum (LGM), ice covered much of the Southern Alps and extended to the coastal lowlands of the central west coast of the South Island. New Zealand's small size and lack of large ice sheets at the glacial maximum allowed vegetation to react quickly to climatic change, with a minimum of periglacial effects from retreating ice sheets or long migrational delays in vegetation recolonization. Pleistocene pollen records in New Zealand are well studied, resulting in reconstructions of the islands' vegetation from the LGM through the present. In the South Island, pollen records indicate that forest was uncommon and most likely consisted of small patches of *Nothofagus* or *Libocedrus* trees, plus small stands of podocarps. A major increase in arboreal pollen at localities across the South Island occurred starting 10 500 years ago, and over the next thousand years, the entire landmass except the dry southeastern interior basins became covered with tall podocarp-hardwood forest (McGlone *et al*. 1993). Today, New Zealand's forests are composed of four broad floristic components: podocarps, hardwoods, *Nothofagus* (the southern beech), and *Agathis australis* Salisb. (kauri). New Zealand's Cyphophthalmi are known from all of these forest types, although a survey of museum collection records and our own fieldwork experiences indicate that where these animals are found, they are most abundant in *Nothofagus* forests and least abundant in the kauri forests of the northernmost peninsula of the North Island. It is reasonable to infer that as *Nothofagus* forests expanded in the postglacial period, Cyphophthalmi populations expanded in number and range, and that in the case of *Aoraki*

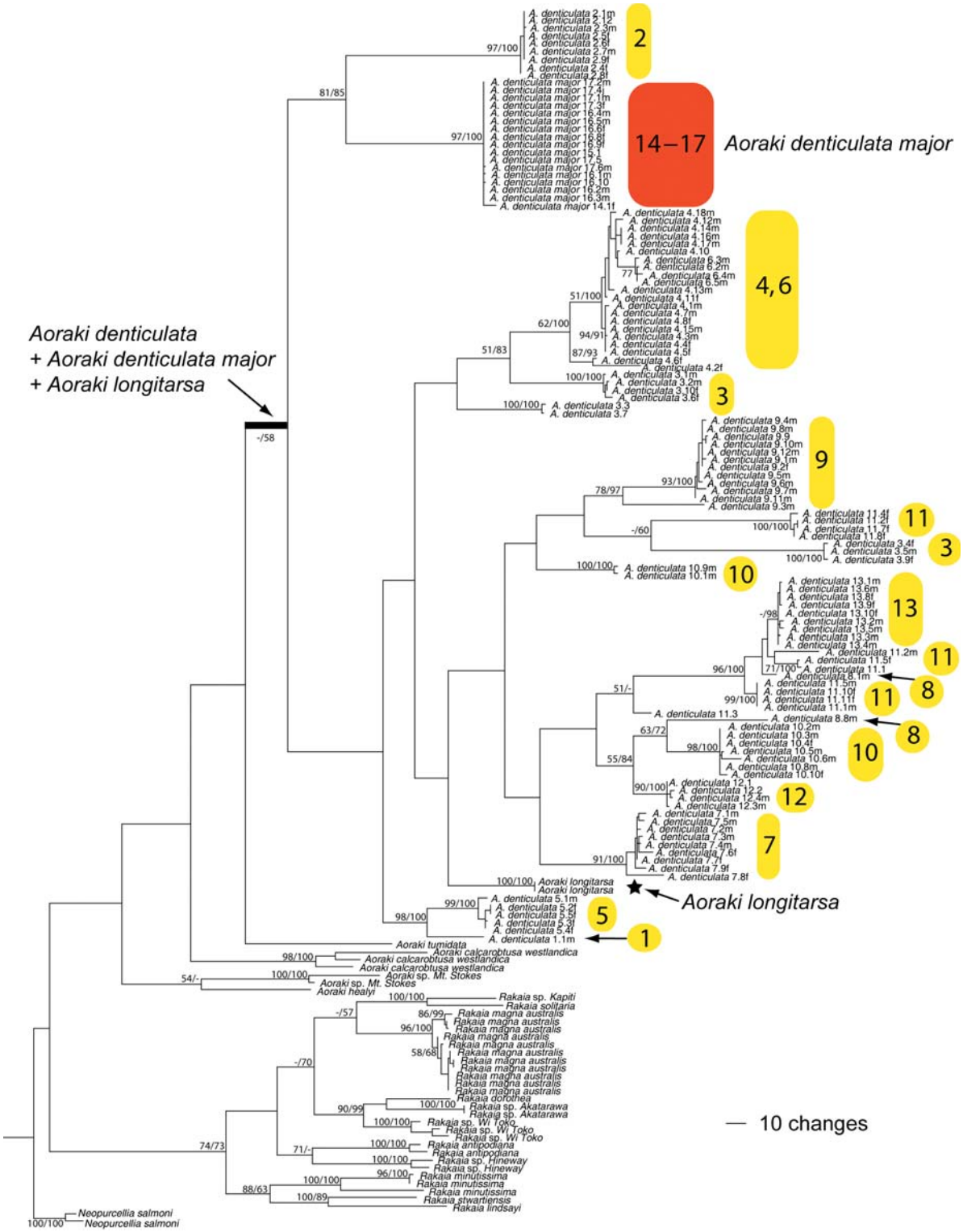

Fig. 4 Phylogeographical relationships of *Aoraki denticulata denticulata, Aoraki denticulata major,* and outgroup taxa. Numbers and colours correspond to the coloured points in Fig. 1: Yellow boxes indicate *A. denticulata denticulata*, the red box indicates *A. denticulata major*, and *Aoraki longitarsa* is marked with a star. The symbol 'm' indicates male, 'f' indicates female, and 'j' indicates juvenile. Maximum-likelihood tree constructed with the GTR + I + Γ model (Tavaré 1986; Rodríguez *et al*. 1990; Yang *et al*. 1994). –Log L = 13668.03717. Numbers on branches indicate bootstrap support (maximum likelihood/parsimony) when above 50%. A dash indicates value lesser than 50%. Support values from some very short internal branches are omitted.

denticulata, such postglacial expansion could have resulted in the admixture of haplotypes from incipient species.

Population genetics and phylogeography of A. denticulata

The degree of genetic differentiation within *A. denticulata denticulata* is remarkable. The only other arthropod known to us to have such large intraspecies divergences is the intertidal copepod *Tigriopus californicus*, which has COI divergences up to 23% (Edmands 2001). In other groups, intraspecies divergences in COI have been reported to be as high as 10% in New Zealand freshwater isopods (McGaughran *et al*. 2006), 7.6% in New Zealand's alpine scree weta (Trewick *et al*. 2000), 5.4% in moths (Brown *et al*. 1994), and 3.8% in beetles (Funk *et al*. 1995), to mention a few of them. In contrast, the highest average genetic distance seen among populations of *A. denticulata denticulata* was 19.2%. AMOVA indicated that the majority of variation across *A. denticulata* occurred between populations rather than within populations (Table 4); however, genetic distances within populations of *A. denticulata denticulata* were also remarkably high in some cases, e.g. 16.4% at locality 8 (with two haplotypes sampled). Haplotypic diversity, which reflects the number of haplotypes in a population without regard for genetic divergences, was similarly high within populations with several localities yielding a unique haplotype for every individual sequenced, resulting in *h* of 1.00 (Table 1; Appendix II).

New Zealand Cyphophthalmi are members of three distinct lineages in the family Pettalidae, a monophyletic group with temperate Gondwanan distribution, and their presence in New Zealand is undoubtedly due to vicariance rather than dispersal (Boyer & Giribet 2007). Therefore, these lineages must be at least 80 Ma, the age of separation of New Zealand from the rest of Gondwana. Without fossil evidence or clear vicariance events within the New Zealand clades, one approach to dating the divergences within *A. denticulata denticulata* is to apply divergence rates from other arthropods, although this may be difficult if *A. denticulata denticulata* has accelerated rates of COI evolution, as postulated earlier. A 1.5% COI divergence (pairwise distance) per million years has been calculated for beetles, and 2.3% per million years for arthropods in general (Brower 1994; Farrell 2001; Trewick & Wallis 2001). Using these estimates, the range of ages of average divergences of haplotypes from different populations within *A. denticulata denticulata* (not including the closely related *A. denticulata major* populations) is 1.68–12.82 Ma or 1.16–8.36 Ma (2.67 to 19.23% sequence divergence, pairwise distance). Considering such rates of COI evolution, this indicates major divergences occurring before and during Pliocene mountain-building (5–2 Ma), clearly postdating the Oligocene drowning period (approximately 37–20 Ma) and fault-mediated displacement (approximately 25–20 Ma) and predating Pleistocene glaciation (approximately 20 000 years ago) in New Zealand. However, if this taxon does exhibit an accelerated rate of COI evolution, as the data suggest, the divergence date could be significantly more recent, rendering Pleistocene glaciation a plausible cause of the remarkable diversity observed in this species. Bond *et al*. (2001) report an accelerated rate of evolution for 16S rRNA in a trapdoor spider, suggesting that a rapid rate of evolution in mitochondrial genes may be common in arachnids. It is important to note that proportional distances were not corrected for homoplasy, adding to the conservative nature of our estimates of divergence time. These estimates remain speculative, as no calibration points exist for the New Zealand pettalids, but they clearly show that the Oligocene drowning episode had no role in the diversification of *A. denticulata*.

Although the data contain little information concerning the relationships among the different populations studied, the group of individuals from locality 2 (Pelorus Bridge) was consistently found to be closely related to the *A. denticulata major* group from localities 14–17 (Arthur's Pass) (Fig. 4). While these populations are widely separated (Fig. 1), they are two of the easternmost populations in the group relative to the main axis of the Southern Alps, a distribution consistent with either Pliocene mountain building or subsequent Pleistocene glaciation driving diversification within this species. Although *A. denticulata* is known from other eastern localities in Marlborough (Fig. 1), extensive collecting efforts at these localities in 2003 and 2006 yielded no specimens.

Relationships of A. denticulata *to other New Zealand Cyphophthalmi*

The genus *Aoraki* is found in both the South Island and North Island of New Zealand, from Aoraki/Mount Cook to Cuvier Island. Previous analyses have found the genus to be unequivocally monophyletic (Boyer & Giribet 2007). As in this study, *A. denticulata denticulata*, *A. denticulata major*, and *A. longitarsa* are found to form a clade sister to the *Aoraki* species *A. calcarobtusa westlandica* and *A. tumidata* plus a species not included here, *A. granulosa* (unpublished data), an unexpected finding (Fig. 4). Morphologically, *A. longitarsa* is clearly related to the rest of the species in the genus, and it has a protuberance at the base of the adenostyle — a secretory organ on the tarsus IV of male adult specimens (see Fig. 3) — similar to that seen in the males of *A. denticulata*, but a particularly close relationship with *A. denticulata* is not self-evident. Most strikingly, the scopulae on the male anal plate of *A. longitarsa* are radically different from those seen in *A. denticulata* (Fig. 2). *A. longitarsa* is comparable in size to *A. denticulata major*, with the two holotype males measuring 2.45 mm and 2.63 mm in length, respectively, but it is considerably larger than the 2 mm long *A. denticulata denticulata* (Forster 1948, 1952).

Geographically, *A. longitarsa* occurs at the southernmost range of the genus and is the only species of *Aoraki* found exclusively to the east of the main axis of the Southern Alps (Fig. 1). *A. longitarsa* is known only from one locality in Aoraki/Mount Cook National Park: Governor's Bush, a tiny patch of beech forest measuring approximately 2 × 4 km. Governor's Bush is extremely isolated from other forests, bounded by the Hooker and Mueller glaciers to the north and west, by Mount Sealy to the southwest, and by the Tasman River to the east. To our knowledge, *A. longitarsa* has been collected only twice: once by J. T. Salmon prior to 1952, and once by S. Boyer, G. Edgecombe, and G. Giribet on 14 January 2006.

There are many possible causes for the type of species paraphyly seen in *A. denticulata* with respect to *A. longitarsa* (Funk & Omland 2003). For example, it is possible that this is a case of peripatric speciation in which a particularly small and isolated population from the *A. denticulata* assemblage has diverged drastically in morphology. Another possibility is that lineage sorting has been incomplete with respect to COI for *A. denticulata* and *A. longitarsa*. Unpublished data from the nuclear markers 18S rRNA and 28S rRNA, including six individuals from *A. denticulata*, indicate that *A. denticulata denticulata, A. denticulata major,* and *A. longitarsa* do indeed form a clade. However, these slow-evolving genes are not able to resolve the relationships among these morphospecies, and it should be emphasized that the nesting of *A. longitarsa* within *A. denticulata* found with the present COI data set is not strongly supported in bootstrap analyses (bootstrap < 50% in likelihood analysis and 58% in parsimony analysis). Therefore, although this result is intriguing, additional data will be necessary to resolve the relationship of *A. denticulata* and *A. longitarsa*, respectively, the most widespread and most isolated species of Cyphophthalmi in New Zealand.

Broader implications: DNA taxonomy and the barcoding of life

Although DNA taxonomy and barcoding are not synonyms — DNA taxonomy proposes an alternative framework to current taxonomic practice, while DNA barcoding mostly aims to aid taxonomy with easier identifications — an increasing portion of the scientific community is participating in broad barcoding initiatives (e.g. Hebert *et al*. 2003, 2004; Kelly *et al*. 2007; Seifert *et al*. 2007). The results of this study are clearly relevant for the debate on barcoding, and argue against using a simplistic approach for identifying species.

While the animals identified as *A. denticulata denticulata* in this study form a coherent morphospecies, they have sometimes hugely different (up to 19.2%) COI sequences. This result speaks to one of the major concerns about the use of DNA barcoding in species identification, the issue of whether to use a distance-based cut-off for species delimitation or whether to apply a tree-based approach (DeSalle *et al*. 2005) — consistent with the phylogenetic species concept. The intraspecific divergences within *A. denticulata* are larger than many of the interspecific differences seen in this study; for example, the branch lengths among the various species in the genus *Rakaia* are much smaller than those seen within *A. denticulata* although *Rakaia* has a geographical distribution spanning most of New Zealand from Stewart Island, off the southern coast of the South Island, to Raetihi in the central part of the North Island.

One suggested rule of thumb for DNA taxonomy is to distinguish species by a cut-off of 10 times the distance observed within species (Hebert *et al*. 2004). This approach to species identification has failed in tests carried out using various species of Diptera (Meier *et al*. 2006) and this rule would clearly be impossible to implement in the genus *Aoraki*. For example, within *A. denticulata* from locality 11, individuals differ by an average of 16.4% in their COI sequence (Table 3), despite forming part of the same clade not affected by the disruption of monophyly by *A. longitarsa*. In each of these cases, we examined male vouchers from the main 'COI groups' for the locality in question (e.g. Figs 2 and 3) and found them to be identical to each other morphologically and unequivocal members of *A. denticulata denticulata*. While it is conceivable that some of the geographically widely separated populations within *A. denticulata denticulata* represent cryptic species, it is difficult to imagine that morphologically identical individuals from a single sample at a unique geographical point are not conspecific, regardless of the very large differences seen within their DNA 'barcodes'. Without detailed ecological study, it is not possible to rule out morphologically identical sympatric species isolated by physiology or chemical ecology, but it is hard to believe that almost every sampled locality would host at least one, if not two, cryptic species of *A. denticulata denticulata*.

Although the degree of COI divergence within *A. denticulata* is remarkable when compared with what is seen in other arthropods, it should be noted that this study generally supports the utility of COI in reconstructing phylogeny in New Zealand Cyphophthalmi. With the exception of *A. denticulata*, our analyses retrieved monophyly of every species and subspecies represented by multiple specimens: *A. calcarobtusa westlandica, A. longitarsa, Aoraki* sp. from Mount Stokes, *Neopurcellia salmoni, Rakaia antipodiana, R. magna australis, R. minutissima*, *Rakaia* sp. from Akatarawa, *Rakaia* sp. from Hineway, and *Rakaia* sp. from Wi Toko. In addition, despite the particularly fast-evolving nature of COI in Cyphophthalmi — a phenomenon reported for other families within the suborder, including Sironidae (Boyer *et al*. 2005) and Stylocellidae (Schwendinger & Giribet 2005) — monophyly of each genus was recovered.

Conclusions

This study represents the first detailed investigation of genetic structure from any species of Opiliones in general and Cyphophthalmi in particular. From a taxonomic point of view, this study confirms the described subspecies *Aoraki denticulata major* as a monophyletic entity. Within the unusually widespread subspecies *Aoraki denticulata denticulata*, most populations are resolved as monophyletic lineages with high support, but the species as a whole appears as a paraphyletic group with respect to *A. denticulata major* and *Aoraki longitarsa*.

Consistent with inferences from known species distribution patterns of Cyphophthalmi, and the large genetic differentiation within and among populations of *A. denticulata denticulata*, we conclude that little genetic exchange occurs among populations from different localities. That is, these animals are spectacularly poor or slow dispersers. As a consequence, they are excellent subjects for biogeographical studies, as their genetic structure is able to reflect both evolutionary and geological patterns. We cannot fully dismiss the hypothesis of the presence of some cryptic species within the conserved *A. denticulata* morphotype. However, the patterns of genetic differentiation seen within *A. denticulata* are compatible with a model in which this extremely old species was deeply subdivided by the Pliocene uplift of the Southern Alps or Pleistocene glaciation, and these events, likely coupled with an accelerated rate of molecular evolution in COI, resulted in the exceptional amount of genetic diversity observed within *A. denticulata* today.

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Appendix I

Detailed collection information for specimens included in this study. Numbers in parentheses following locality names correspond to locality numbers in Fig. 1. DNA Voucher numbers are deposited at the Museum of Comparative Zoology, Harvard University. *N*, number of specimens

Species	Locality name	Coordinates	Vouchers	*N*	GenBank Accession no.
Aoraki denticulata	Totaranui (1)	40°49′57″S, 172°58′08″E	DNA101296	1	DQ992312
Aoraki denticulata	Pelorus Bridge (2)	41°18′03″S, 173°34′35″E	DNA101811	9	DQ992194–202
Aoraki denticulata	Karamea Bight (3)	41°31′41″S, 172°01′16″E	DNA100948	9	DQ992241–246, DQ992288–290
Aoraki denticulata	Longford (4)	41°47′02″S, 172°22′09″E	DNA100941, DNA101821	17	DQ992220-236
Aoraki denticulata	Lake Rotoiti (5)	41°48′29″S, 172°50′01″E	DNA100961	5	DQ992295–299
Aoraki denticulata	St Arnaud (6)	41°46′07″S, 172°56′53″E	DNA101845	4	DQ992237–240
Aoraki denticulata	10 Mile Creek (7)	41°50′09″S, 171°40′36″E	DNA101825	8	DQ992265–673
Aoraki denticulata	Bumbrae (8)	41°57′59″S, 172°13′51″E	DNA101826	2	DQ992275–281
Aoraki denticulata	Punakaiki (9)	42°05′34″S, 171°20′27″E	DNA100955, DNA101842	18	DQ992309–311, DQ992247–255
Aoraki denticulata	Reefton(10)	42°09′23″S, 171°47′34″E	DNA100964	9	DQ992300–308
Aoraki denticulata	RahuSc. Res. (11)	42°19′56″S, 172°10′07″E	DNA101124, DNA101828	12	DQ992274, DQ992277–280, DQ992282–287
Aoraki denticulata	Ahaura Road (12)	42°22′50″S, 171°39′03″E	DNA101831	4	DQ992291–295
Aoraki denticulata	Mitchell (13)	42°31′19″S, 171°24′03″E	DNA101823	9	DQ992256–264
Aoraki denticulata major	Otira(14)	42°50′03″S, 171°33′45″E	DNA101835	1	DQ992219
Aoraki denticulata major	Bealey Gorge (15)	42°55′11″S, 171°33′28″E	DNA101836	1	DQ992212
Aoraki denticulata major	Greney's Shelter (16)	42°59′03″S, 171°35′26″E	DNA101837	9	DQ992215-218, DQ992207–211
Aoraki denticulata major	Cass (17)	43°02′03″S, 171°45′53″E	DNA100959, DNA101839	6	DQ992203-206, DQ992213–214
A. calcarobtusa westlandica	Longford (4)	41°47′02″S, 172°22′09″E	DNA101129	1	DQ992316
A. calcarobtusa westlandica	Truman Track	41°47′02″S, 172°22′09″E	DNA101125	1	DQ992315
Aoraki healyi	Mt. Stokes	41°05′12″S, 174°08′18″E	DNA100940	1	DQ992321
Aoraki longitarsa	Aoraki Mount Cook	43°44′12″S, 170°05′32″E	DNA101806	2	DQ992313–314
Aoraki tumidata	Paengaroa	unavailable	MONZ-DOC	1	DQ992318
Aoraki sp. *Mt. Stokes*	Mount Stokes	41°05′14″S, 174°08′14″E	DNA101126, DNA101809	2	DQ992319–320
Neopurcellia salmoni	Totara Saddle	42°55′05″S, 170°52′20″E	DNA101832	1	DQ992323
Neopurcellia salmoni	Lake Kaniere	42°48′13″S, 171°07′44″E	DNA101834	1	DQ992324
Rakaia antipodiana	Cass (17)	43°02′03″S, 171°45′53″E	DNA101127	1	DQ992329
Rakaia antipodiana	Awa Awa Rata	unavailable	MONZ-DOC	1	DQ992330
Rakaia dorothea	Days Bay	41°16′54″S, 174°54′35″E	DNA100943	1	DQ992331
Rakaia florensis	Totaranui (1)	40°49′57″S, 172°58′08″E	DNA101295	1	DQ992325
Rakaia lindsayi	Stewart Island	43°02′03″S, 171°45′53″E	DNA101128	1	DQ992352
R. magna australis	St James Walkway	42°19′56″S, 172°10′17″E	DNA100962	1	DQ992333
R. magna australis	RahuSc. Res. (11)	42°22′38″S, 172°24′12″E	DNA100963	1	DQ992334
R. magna australis	Springs Junction	42°17′26″S, 172°00′58″E	DNA100965	1	DQ992335
R. magna australis	Boyle Village	42°29′35″S, 172°23′17″E	DNA101830	8	DQ992336–342
Rakaia minutissima	Raetihi	39°24′59″S, 175°13′07″E	DNA101291	1	DQ992326
Rakaia minutissima	Totaranui (1)	40°49′57″S, 172°58′08″E	DNA101295	1	DQ992327
Rakaia minutissima	Canaan Road	40°56′35″S, 172°53′30″E	DNA101813	1	DQ992328
Rakaia solitaria	Opouawe Gully	41°28′01″S, 175°26′56″E	DNA101294	1	DQ992343
Rakaia stewartiensis	Stewart Island	46°53′36″S, 168°06′14″E	DNA101944	1	DQ992351
Rakaia sp. *Kapiti*	Kapiti island	40°51′07″S, 175°55′56″E	DNA101293	1	DQ992322
Rakaia sp. *Akatarawa*	Akatarawa	40°58′33″S, 175°07′02″E	DNA101297	1	DQ992344
Rakaia sp. *Akatarawa*	Akatarawa	40°56′45″S, 175°06′35″E	DNA101298	1	DQ992345
Rakaia sp. *Hineway*	Hineway Reserve	43°48′31″S, 173°01′17″E	DNA100958	2	DQ992349–350
Rakaia sp. *Wi Toko*	Wi Toko Scenic Res.	41°09′27″S, 175°01′18″E	DNA100954	1	DQ992348
Rakaia sp. *Wi Toko*	Otaki Forks	40°52′04″S, 175°14′04″E	DNA101299	1	DQ992347
Rakaia sp. *Wi Toko*	Shannon	40°34′49″S, 175°29′20″E	DNA101300	1	DQ992346

Appendix II

Absolute haplotype frequencies for each population studied. Localities and frequencies in regular type indicate *Aoraki denticulata denticulata* populations, while localities and frequencies in bold (H80–H84) indicate *A. d. major* populations.

	Localities																
Haplotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	**14**	**15**	**16**	**17**
H1	1																
H2		6															
H3		1															
H4		2															
H5			1														
H6			1														
H7			1														
H8			1														
H9			1														
H10			1														
H11			1														
H12			1														
H13			1														
H14				1													
H15				1													
H16				1													
H17				3													
H18				1													
H19				5													
H20				1													
H21				1													
H22				1													
H23				1													
H24				1													
H25				1													
H26					1												
H27					2												
H28					1												
H29					1												
H30						1											
H31						1											
H32						1											
H33						1											
H34							1										
H35							1										
H36							1										
H37							1										
H38							1										
H39							1										
H40							1										
H41							1										
H42							1										
H43								1									
H44								1									
H45									1								
H46									1								
H47									1								
H48									1								
H49									3								
H50									1								
H51									1								
H52									1								
H53									1								

Appendix II *Continued*

	Localities																
Haplotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	**14**	**15**	**16**	**17**
H54									1								
H55										4							
H56										1							
H57										1							
H58										1							
H59										1							
H60										1							
H61											1						
H62											1						
H63											4						
H64											1						
H65											1						
H66											1						
H67											2						
H68											1						
H69												1					
H70												1					
H71												1					
H72												1					
H73													1				
H74													2				
H75													1				
H76													1				
H77													1				
H78													2				
H79													1				
H80														1			
H81															**1**	**7**	**5**
H82																**1**	
H83																**1**	
H84																	**1**