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ORIGINAL ARTICLE

Genetic divergences pre-date Pleistocene glacial cycles in the New Zealand speckled skink, *Oligosoma infrapunctatum*

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ABSTRACT

Aim To examine the hypothesis raised by Graham S. Hardy that Pleistocene glacial cycles suffice to explain divergence among lineages within the endemic New Zealand speckled skink, *Oligosoma infrapunctatum* Boulenger.

Location Populations were sampled from across the entire range of the species, on the North and South Islands of New Zealand.

Methods We sequenced the mitochondrial genes *ND2* (550 bp), *ND4* + tRNAs (773 bp) and *cytochrome b* (610 bp) of 45 individuals from 21 locations. Maximum likelihood, maximum parsimony and Bayesian methods were used for phylogenetic reconstruction. The Shimodaira–Hasegawa test was used to examine hypotheses about the taxonomic status of morphologically distinctive populations.

Results Our analysis revealed four strongly supported clades within *O. infrapunctatum*. Clades were largely allopatric, except on the west coast of the South Island, where representatives from all four clades were found. Divergences among lineages within the species were extremely deep, reaching over 5%. Two contrasting phylogeographical patterns are evident within *O. infrapunctatum*.

Main conclusions The deep genetic divisions we found suggest that *O. infrapunctatum* is a complex of cryptic species which diverged in the Pliocene, contrary to the existing Pleistocene-based hypothesis. Although Pleistocene glacial cycles do not underlie major divergences within this species, they may be responsible for the shallower phylogeographical patterns that are found within *O. infrapunctatum*, which include a radiation of haplotypes in the Nelson and Westland regions.

Keywords
Cytochrome *b*, glacial refugia, historical biogeography, lizard, mtDNA, ND2, ND4, New Zealand, phylogeography, Pliocene divergence.

INTRODUCTION

It is clear that Pleistocene glacial cycles have left their imprint on patterns of genetic diversity worldwide (reviewed in Markgraf *et al.*, 1995; Hewitt, 1996, 2000, 2004). However, the relative importance of late Pleistocene glacial cycles in driving divergence and speciation is controversial (e.g. Zink & Slowinski, 1995; Klicka & Zink, 1997, 1999; Arbogast & Slowinski, 1998; Avise & Walker, 1998; Avise *et al.*, 1998; Moritz *et al.*, 2000; Johnson & Cicero, 2004; Weir & Schluter, 2004; Zink *et al.*, 2004; Zink & Klicka, 2006). In particular, large genetic distances between sister taxa in many groups are thought to suggest Pliocene or early Pleistocene divergence, rather than divergence in the late Pleistocene (Klicka & Zink, 1997; Moritz *et al.*, 2000). Recently reinvigorated debate focuses on whether late Pleistocene glacial cycles caused an explosion in speciation (Johnson & Cicero, 2004), or whether both Pliocene and Pleistocene processes have contributed significantly to the diversity of extant species (Zink & Klicka, 2006). However, a pluralist view, citing roles for both Pliocene and Pleistocene processes in speciation, has been advocated in a recent review of the debate (Lovette, 2005; see also Klicka & Zink, 1997; Avise & Walker, 1998).

The fragmented, oceanic nature of Southern Hemisphere systems provides another diverse set of examples with which to understand the effect of Pleistocene glacial cycles on speciation (Markgraf *et al.*, 1995). In contrast with Northern Hemisphere systems, glacial cycles in most Southern Hemisphere systems were not marked by the appearance of large continental ice sheets, and it is thought that glacial climate change was milder in most southern systems than in northern systems (Markgraf *et al.*, 1995). Glacial cycles in New Zealand commenced in the late Pliocene, occurring largely in time (within 2–3 kyr) with ice-sheet advances in the Northern Hemisphere (Pillans, 1991). New Zealand's South Island was extensively glaciated during glacial periods, with the formation of a complex of extensive valley glaciers and ice fields along the Southern Alps and some isolated mountains in the north-west (Suggate, 1990). Additionally, tectonic movement and worldwide changes in sea level caused large-scale coastline changes throughout the Pleistocene. In particular, an intermittent land bridge formed across Cook Strait during some or all glacial periods, joining the North and South Islands and potentially providing new migratory routes for taxa retreating in response to climate change (Newnham *et al.*, 1999).

The Pliocene history of New Zealand is also complex. In particular, all of New Zealand's mountains, except for volcanoes, are the result of Pliocene tectonism (Gage, 1980). The Southern Alps, which are a result of this on-going cycle of tectonism and run most of the length of the South Island, posed an immense barrier to dispersal, created novel alpine environments and partitioned climate and rainfall east to west (Chinn & Gemmell, 2004; Trewick & Morgan-Richards, 2005). Both Pleistocene and Pliocene processes could thus be expected *a priori* to have had impacts on modern-day patterns of distribution and diversity in New Zealand taxa. Echoing the Pleistocene–Pliocene debate in northern systems, there has been considerable debate within New Zealand biogeography about whether various pre-Pleistocene tectonic processes or Pleistocene glacial cycles underlie biogeographical patterns within the archipelago (e.g. McGlone, 1985; Wardle, 1988; McGlone *et al.*, 2001; Trewick & Wallis, 2001; Heads & Craw, 2004).

The New Zealand skink fauna is diverse and comprises at least 28 species classified into two endemic genera: *Oligosoma* and *Cyclodina* (Daugherty *et al.*, 1994). *Oligosoma infrapunctatum* Boulenger is a widespread but patchily distributed skink with a distribution spanning regions of biogeographical interest within New Zealand, including Cook Strait and a putative former glacial refugium in the Nelson region (Fig. 1b). On the South Island, it occurs only on the western side of the Southern Alps. *Oligosoma infrapunctatum* occurs

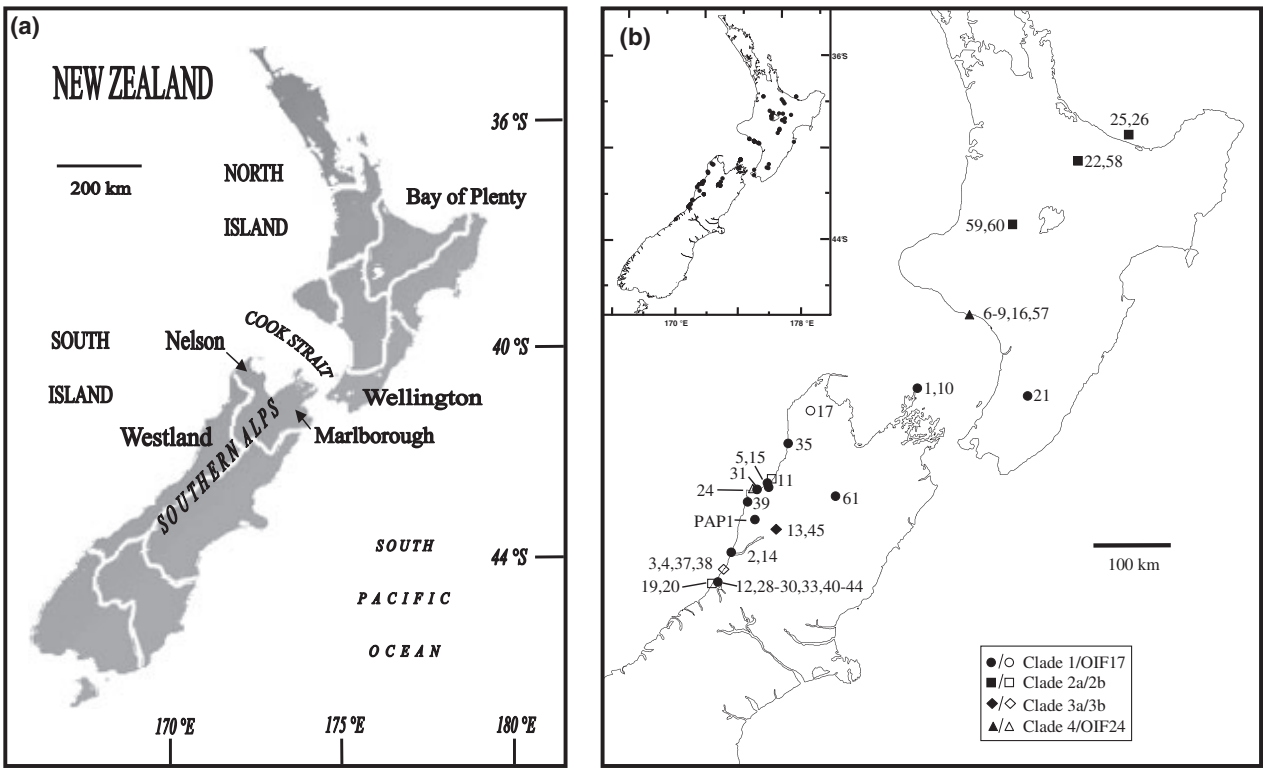

Figure 1 (a) Map showing regions of New Zealand. (b) Map showing the sampling localities of the tissue samples for the *Oligosoma infrapunctatum* Boulenger samples in Table 1. The inset shows the distribution of *O. infrapunctatum* based on the BioWeb Herpetofauna Database 2006, New Zealand Department of Conservation (see Methods).

in open forest, scrubland and tussock grassland from sea level to the subalpine zone (Gill & Whitaker, 2001), on boulder beaches in the Westland region of the South Island (Whitaker & Lyall, 2004) and occasionally in farmland and other modified habitats (Whitaker & Lyall, 2004). Subfossil evidence indicates that the species was once distributed more widely in the North Island, particularly in the Northland region (Worthy, 1991). *Oligosoma infrapunctatum* shows considerable geographical variation in its body size, colour and pattern (Hardy, 1977; Whitaker & Lyall, 2004). For example, maximum body size (snout–vent length, SVL) varies among populations, from 75 mm SVL (Whale Island population) to 106 mm SVL (Stephens Island population) (Hardy, 1977; Towns *et al.*, 2002). Those from Westland populations in the South Island fall at the lower end of this range, reaching a maximum body size of 80 mm SVL (Whitaker & Lyall, 2004).

Geographical variation within the species contributes to several interesting taxonomic questions. In particular, the Westland region harbours several morphologically distinctive populations of uncertain taxonomic status – *Oligosoma* sp. 'Paparoa', *Oligosoma* sp. 'Denniston' and *O. infrapunctatum* 'Chesterfield' (Whitaker & Lyall, 2004). Allozyme data have proven equivocal as to their taxonomic distinctiveness (Miller, 1999; C.H.D., unpublished data). Likewise, the taxonomic distinctiveness of a recently discovered population in the Wanganui region of the North Island (*O. infrapunctatum* 'Southern North Island') is uncertain.

It has been proposed that the current distribution of *O. infrapunctatum* and its patterns of variation are the result of the impacts of Pleistocene glacial cycles, specifically migration across land bridges, and range shifts into and out of a glacial refugium proposed to have existed in the Nelson region (Hardy, 1977). Here we re-examine Hardy's (1977) hypothesis that Pleistocene processes suffice to explain distribution and genetic patterns within *O. infrapunctatum*, by using mitochondrial DNA (mtDNA) sequence data (*ND2*, *ND4* and *cytb*), additional samples from populations unknown at the time of Hardy's (1977) study, and a phylogeographical framework.

METHODS

Sampling

Samples were obtained from the National Frozen Tissue Collection (NFTC; Victoria University of Wellington, New Zealand) and from ethanol-preserved museum specimens from Te Papa (National Museum of New Zealand, Wellington), for sites covering the entire known range of *O. infrapunctatum*, based on the BioWeb Herpetofauna Database 2006, New Zealand Department of Conservation (information on the data base is available from http://www.doc.govt.nz/templates/page.aspx?id=33158, last accessed 22 October 2007) (Fig. 1, Table 1). We also obtained five field-collected samples. Two outgroup species, *Oligosoma otagense* (Otago skink) and *Oligosoma acrinasum* (Fiordland skink), were chosen based on a wider phylogenetic study of endemic New Zealand skinks (D.G.C., C.H.D. and P.A.R., unpublished data).

DNA extraction, amplification and sequencing

We extracted total genomic DNA using a modified phenol and chloroform protocol (Sambrook *et al.*, 1989) followed by ethanol precipitation. We used polymerase chain reaction (PCR) with the primers listed in Table 2 to amplify fragments from three mitochondrial loci for each sample: *ND2*, *ND4* plus *tRNA-His* and part of *tRNA-Ser,* and *cytochrome b*. Previous work using *ND2, ND4* and *cytochrome b* (Chapple & Keogh, 2004; Chapple *et al.*, 2004, 2005; Keogh *et al.*, 2005) has shown that these mitochondrial regions are sufficiently variable to be useful for intraspecific studies in squamate reptiles. PCR and sequencing were conducted as outlined in Greaves *et al.* (2007).

Phylogenetic analysis

Sequence data were edited manually using ContigExpress in Vector NTI Advance 9.1.0 (Invitrogen, Carlsbad, USA) then aligned using ClustalX (Thompson *et al.*, 1997) executed in mega 3.1 (Kumar *et al.*, 2004). We translated all sequences to confirm that none contained premature stop codons. Sequence data were submitted to GenBank under the accession numbers provided in Table 1.

A partition-homogeneity test executed in paup* 4.0b10 (Swofford, 1998) was used to test for discordance (100 replicates; $P = 0.57$). Since no significant discordance was found, we concatenated the sequences for each individual to create a single data set. To determine the most appropriate model of evolution for our data set, we generated log-likelihood scores for the data set using paup* and then used those scores to conduct a hierarchical likelihood ratio test (hLRT) in Modeltest 3.7 (Posada & Crandall, 1998). Modeltest was also used to estimate base frequencies, substitution rates, the proportion of invariable sites and the among-site substitution rate variation. These values were then used as settings in paup* to generate a maximum likelihood (ML) phylogenetic tree. MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) was used to complete Bayesian analysis. We ran the full analysis twice, using four Markov chains per run. We ran the chains for 1 million generations, to ensure sufficient sampling of tree space. The chain was sampled every 100 generations to obtain 10,000 sampled trees. The first 25% of sampled trees was discarded as the burn-in phase and the last 7500 trees were used to estimate the Bayesian posterior probabilities. Bootstrap values and Bayesian prior probabilities were used to assess branch support. Our data set was too large to do ML bootstraps, so we performed a parsimony bootstrap analysis. We considered branches supported by bootstrap values of 70% or

Table 1 Museum registration numbers and sampling localities for samples used in this study.

Sample	Species	Museum tissue code	Locality	GenBank accession numbers		
				ND2	*ND4*	*Cytb*
OIF1	*Oligosoma infrapunctatum* Boulenger	CD545	Stephens Island	EF033050	EF033058	EF071066
OIF2	*O. infrapunctatum*	FT3749	Cobden Beach	EF033051	EF033059	EF071067
OIF3	'Chesterfield' *O. infrapunctatum*	FT3214	Chesterfield	EF070984	EF071024	EF071068
OIF4	'Chesterfield' *O. infrapunctatum*	FT3215	Chesterfield	EF070985	EF071025	EF071069
OIF5	'Denniston' *O. infrapunctatum*	FT6272	Denniston	EF070986	EF071026	EF071070
OIF6	'South North Island' *O. infrapunctatum*	(field collected)	Waiinu Beach	EF070987	EF071027	EF071071
OIF7	'South North Island' *O. infrapunctatum*	(field collected)	Waiinu Beach	EF070988	EF071028	EF071072
OIF8	'South North Island' *O. infrapunctatum*	(field collected)	Waiinu Beach	EF070989	EF071029	EF071073
OIF9	'South North Island' *O. infrapunctatum*	(field collected)	Waiinu Beach	EF070990	EF071030	EF071074
OIF10	*O. infrapunctatum*	CD531	Stephens Island	EF070991	EF071031	EF071075
OIF11	*O. infrapunctatum*	FT3395	Granity	EF070992	EF071032	EF071076
OIF12	*O. infrapunctatum*	FT3481	Hokitika	EF070993	EF071033	EF071077
OIF13	*O. infrapunctatum*	FT3745	Alborn Mine	EF070994	EF071034	EF071078
OIF14	*O. infrapunctatum*	FT3752	Cobden Beach	EF070995	EF071035	EF071079
OIF15	*O. infrapunctatum*	FT3758	Birchfield Beach	EF070996	EF071036	EF071080
OIF16	*O. infrapunctatum*	FT6269	Waiinu Beach	EF070997	EF071037	EF071081
OIF17	*O. infrapunctatum*	FT6270	Brown Hill, Heaphy Track	EF070998	EF071038	EF071082
OIF19	*O. infrapunctatum*	RE 5235	Hokitika	EF070999	EF071039	EF071083
OIF20	*O. infrapunctatum*	RE 5235	Hokitika	EF071000	EF071040	EF071084
OIF21	*O. infrapunctatum*	RE 5242	Mt Bruce	EF071001	EF071041	EF071085
OIF22	*O. infrapunctatum*	RE 5247	Rotorua	EF071002	EF071042	EF071086
OIF24	*O. infrapunctatum*	FT3004	Westport	EF071003	EF071043	EF071087
OIF25	*O. infrapunctatum*	FT3023	Whale Island	EF071004	EF071044	EF071088
OIF26	*O. infrapunctatum*	FT3024	Whale Island	EF071005	EF071045	EF071089
OIF28	*O. infrapunctatum*	FT3740	Hokitika Cemetery	EF071006	EF071046	EF071090
OIF29	*O. infrapunctatum*	FT3741	Hokitika Cemetery	EF071007	EF071047	EF071091
OIF30	*O. infrapunctatum*	FT3742	Hokitika Cemetery	EF071008	EF071048	EF071092
OIF31	*O. infrapunctatum*	FT3809	Orowaitai Lagoon	EF071009	EF071049	EF071093
OIF33	*O. infrapunctatum*	FT3476	Hokitika	EF071010	EF071050	EF071094
OIF35	*O. infrapunctatum*	FT3768	Oparara Rd, Karamea	EF071012	EF071052	EF071095
OIF37	'Chesterfield' *O. infrapunctatum*	FT3770	Chesterfield	EF071013	EF071053	EF071096
OIF38	'Chesterfield' *O. infrapunctatum*	FT3771	Chesterfield	EF071014	EF071054	EF071097
OIF39	*O. infrapunctatum*	FT3795	Costello Hill, Pakahi Charleston	EF071015	EF071055	EF071098
OIF40	*O. infrapunctatum*	FT3799	Kaniere Rd, Hokitika	EF071016	EF071056	EF071099
OIF41	*O. infrapunctatum*	FT3800	Kaniere Rd, Hokitika	EF071017	EF071057	EF071100
OIF42	*O. infrapunctatum*	FT3801	Kaniere Rd, Hokitika	EF071018	EF071058	EF071101
OIF43	*O. infrapunctatum*	FT3802	Kaniere Rd, Hokitika	EF071019	EF071059	EF071102
OIF44	*O. infrapunctatum*	FT3803	Kaniere Rd, Hokitika	EF071020	EF071060	EF071103
OIF45	*O. infrapunctatum*	FT3747	Alborn Mine	EF071021	EF071061	EF071104
OIF57	'South North Island' *O. infrapunctatum*	(field collected)	Castlecliff	EF071022	EF071062	EF071105
OIF58	*O. infrapunctatum*	CD2531	Ngongataha (Rotorua)	EF175856	EF175860	EF175864
OIF59	*O. infrapunctatum*	CD2532	Taumaranui	EF175857	EF175861	EF175865
OIF60	*O. infrapunctatum*	CD2533	Taumaranui	EF175858	EF175862	EF175866
OIF61	*O. infrapunctatum*	FT3003	St Arnaud	EF175859	EF175863	EF175867
PAP1	'Paparoa' *O. infrapunctatum*	FT3815	Paparoa Ranges	EF071023	EF071063	EF071106
OAC1	*O. acrinasum*	CD826	Fiordland	EF033046	EF033060	EF071064
OOT1	*O. otagense*	CD1053	Central Otago	EF033053	EF033064	EF071065

Museum collections: FT & CD, National Frozen Tissue Collection (NFTC), housed at Victoria University of Wellington, New Zealand; RE, Museum of New Zealand Te Papa Tongarewa.

greater (Hillis & Bull, 1993), and/or posterior probability values greater than or equal to 95% (Wilcox *et al.*, 2002) to be supported by our data, and branches with weaker support were collapsed.

Molecular clock analysis

To estimate the time since the divergence of lineages within *O. infrapunctatum*, we calibrated the evolutionary rate of *ND2* by

Table 2 Oligonucleotide primers used in this study. Values in the '5′ position' refer to the position of the 5′ base of the primer in the complete *Eumeces egregius* mtDNA sequence (Kumazawa & Nishida, 1999).

Mt region	Primer	5′–3′ sequence	5′ position	Source
ND2	L4437	AAGCTTTCGGGCCCATACC	3833	Macey *et al.* (1997)
	ND2r102	CAGCCTAGGTGGGCGATTG	4432	Sadlier *et al.* (2004)
	ND2F-infrapunctatum	GCATGATTYACCGGAAYATGAGACAT	4141	Greaves *et al.* (2007)
	ND2R-infrapunctatum	GGGGCAAGKCCTAGTTTTATGG	4192	Greaves *et al.* (2007)
ND4	ND4I	TGACTACCAAAAGCTCATGTAGAAGC	10771	Forstner *et al.* (1995)
	ND4R-NZ	CCAAGRGTTTTGGTGCCTAAGACC	11670	This study
	tRNA-Leu	TACTTTTACTTGGATTTGCACCA	11691	Forstner *et al.* (1995)
Cytb	mtD25	CCATCCAACATCTCAGCATGATGAAA	14940	Kocher *et al.* (1989)
	SkCytBR	TAGGCAAANARRAAGTAYCAYTCTGG	14202	This study

reanalysing data from Macey *et al.* (1998) for the agamid genus *Laudakia*. Macey *et al.* (1998) calibrated this rate by geological dating of tectonic events on the Iranian Plateau. Their rate has been demonstrated to be consistent (*c.* 1.2–1.4% Myr^{-1}) across several vertebrate groups (fish, amphibians, reptiles; reviewed in Weisrock *et al.*, 2001). Specifically, we recalculated the evolutionary rate for *Laudakia* using only the 550-bp fragment of *ND2* used in the present study (e.g. Smith *et al.*, 2007). We calculated average between-group nucleotide differences (uncorrected) across each of the calibrated nodes from Macey *et al.* (1998) (1.5, 2.5 and 3.5 Ma), plotted them against time and used the slope of the linear regression to calculate a rate of evolution for our 550-bp fragment of *ND2*. This resulted in an evolutionary rate of 1.4% Myr^{-1} (0.7% per lineage Myr^{-1}) and is slightly faster than the rate of 1.3% Myr^{-1} found by Macey *et al.* (1998).

Hypothesis testing

Using the Shimodaira–Hasegawa test in PAUP* (Shimodaira & Hasegawa, 1999) with the resampling log-likelihood method (Kishino *et al.*, 1990), we tested the significance of differences between our ML tree and topologies representing various alternative hypotheses regarding the taxonomic status of morphologically distinctive populations within the *O. infrapunctatum* group. Specifically, we tested the hypothesis that each of these lineages was sister to the group formed by Clade 1, OIF17 and Clade 2.

1. The 'Paparoa skink' (*Oligosoma* sp. 'Paparoa'), is a morphologically distinctive form of *O. infrapunctatum*. It is known from a single specimen only, a heavy-bodied skink (79 mm SVL). It was discovered at an altitude of 1400 m in the Paparoa Ranges, and was presumed to be a new taxon (Miller, 1999; Patterson, 2002; Whitaker & Lyall, 2004).

2. The 'Denniston skink' (*Oligosoma* sp. 'Denniston') is morphologically distinctive but shows strong similarities to *O. infrapunctatum*. It is known from two specimens only, captured on the Denniston Plateau in Westland, and has a body size of 83 mm SVL. It was believed to constitute a new taxon, although detailed knowledge of this population is lacking (Miller, 1999; Whitaker & Lyall, 2004).

3. The 'Chesterfield skink' (*O. infrapunctatum* 'Chesterfield') is morphologically distinctive because of red and pink markings on the tail that are unique among New Zealand skinks. It shows wide variation in body size, and reaches sizes up to 80 mm SVL (Aviss & Lyall, 1995). It was discovered in 1992 in Chesterfield, a collection of farms on the coast in central Westland (Aviss & Lyall, 1995). Allozyme studies have not supported the genetic distinctiveness of the Chesterfield skink from *O. infrapunctatum* (Miller, 1999), but its morphological distinctiveness has led to suggestions that more sensitive genetic testing be conducted (Whitaker & Lyall, 2004).

4. *Oligosoma infrapunctatum* 'Southern North Island' was discovered in 2001 at Waiinu Beach on the southern west coast of the North Island. This skink shows similarities in morphology to both *O. infrapunctatum* and the common skink, *O. nigriplantare*. It is classified as 'nationally endangered' by the New Zealand Department of Conservation (Hitchmough *et al.*, 2007).

RESULTS

Our final data set comprised sequences from 45 individuals from 21 different locations, as well as sequences from two outgroup species. For each individual, we obtained sequences from the mitochondrial loci *ND2* (550 bp), *ND4* + tRNAs (773 bp) and *cytochrome b* (610 bp). After concatenation, the aligned data set contained 1933 characters, of which 473 (24%) were variable and 318 (16%) were parsimony-informative. For the ingroup only, the alignment contained 330 (17%) variable characters of which 270 (14%) were parsimony-informative. Base frequencies were unequal (A = 31%, T = 26%, C = 30%, G = 13%), but a chi-square test confirmed the homogeneity of base frequencies among all sequences (d.f. = 138, $P = 1.00$).

The hLRT conducted in MODELTEST selected the Tamura Nei (TrN) plus gamma shape (+Γ) substitution model as the most appropriate for our data set (–ln $L = 6378.6904$). We used this model to estimate the relative rate of each substitution type, and found a strong bias towards transition substitutions (A↔C = 1.00, A↔G = 23.84, A↔T = 1.00, C↔G = 1.00, C↔T = 11.94, G↔T = 1.00). MODELTEST esti-

mated the gamma shape parameter as 0.2266. The unweighted parsimony, ML and Bayesian analyses recovered the same six clades as well as two divergent lineages each represented by a single sample (OIF17 and OIF24), and which were therefore not assigned to clades. Figure 2 shows the result of ML analysis, with weak branches collapsed (ML –ln L = 6377.13621). The six clades recovered in all analyses were supported by extremely high bootstrap values (100% in all cases) and posterior probabilities (0.99–1.00), but the interrelationships of Clades 1, 2 and 3 were not resolved.

Substantial genetic structure exists within *O. infrapunctatum*. Genetic divergence between representative members of the clades and lineages recovered in our analysis (Fig. 2) ranges between 2.8% and 7.3% (Table 3). The range of genetic distances within clades varies between 0.0% and 2.6% (Table 4). Clade 1 consists of a large number of closely related haplotypes. Genetic distances between haplotypes in Clade 1 range from 0.0% to 1.4%. Populations from this clade are found in the south of the North Island (in one location only), in Cook Strait, in Nelson and throughout coastal Westland south to Hokitika (Figs 1 & 2). This clade contains the morphologically distinctive 'Paparoa' and 'Denniston' skinks. Our topology tests do not support the hypothesis that either of these populations is genetically divergent from the main *O. infrapunctatum* group (Table 5).

The rest of the clades are characterized by much deeper divergences among haplotypes. North and South Island lineages in this part of the tree are not reciprocally monophyletic. Thus, Clade 2 is divided into two divergent subclades, one occurring in Westland on the South Island (Clade 2a; Figs 1 & 2), with the other in the northernmost region of the species distribution, in the Bay of Plenty and central North Island (Clade 2b; Figs 1 & 2). The genetic distance between these clades is 4.4% (Table 3).

Clade 3 contains samples from two locations in Westland, from the distinctive 'Chesterfield' skink (Clade 3a; Figs 1 & 2) and from Alborn Mine (Clade 3b; Figs 1 & 2). The genetic distance between these populations is 2.8%, while the distance between the 'Chesterfield' skink and Clade 1 is 5.7% (Table 3).

Clade 4 contains samples from the distinctive *O. infrapunctatum* 'Southern North Island' population (Figs 1 & 2). The genetic distance between Clade 4 and Clade 1 is 5.0% (Table 3). A sister lineage to Clade 4 is represented by a single sample from the Westland town of Westport, which shows 5.2% genetic distance from Clade 1. Since our phylogenetic

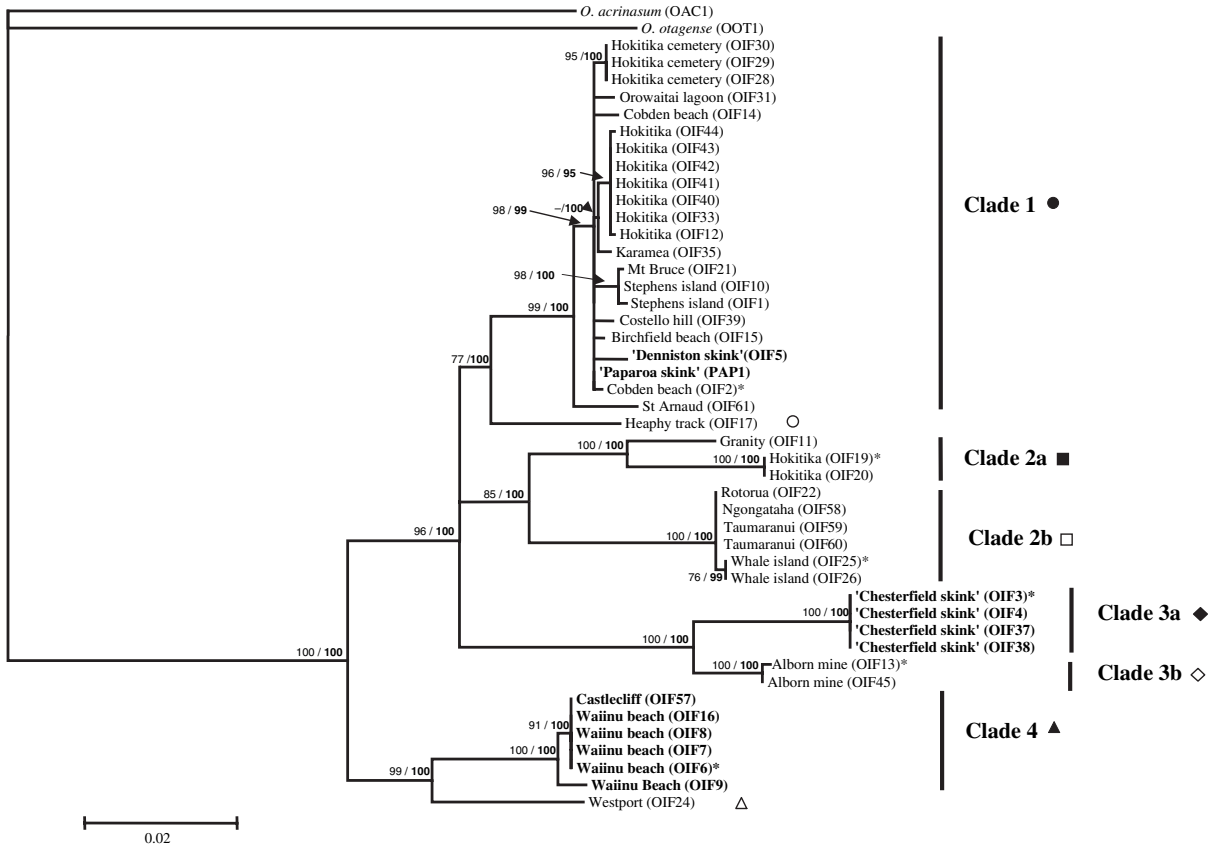

Figure 2 Phylogram from maximum likelihood analysis for *Oligosoma infrapunctatum* based on the combined *ND2*, *ND4* and *cytb* dataset (1933 bp). Parsimony bootstrap values are shown in plain text and Bayesian posterior probabilities are shown in bold. Six clades are identified within *O. infrapunctatum*. Two divergent lineages represented by single samples only, OIF17 (Brown Hill, Heaphy Track) and OIF24 (Westport), have not been assigned to clades.

Table 3 Uncorrected pairwise distance matrix for representatives (marked with asterisks on Fig. 2) of each clade identified in Fig. 2. Distances calculated using the complete data set are below the diagonal. Distances calculated using *ND2* only (for molecular clock analysis) are above the diagonal.

		Clade	1	2	3	4	5	6	7	8	9	10
1	OAC1	Outgroup	–	0.1130	0.1110	0.1150	0.1220	0.1250	0.1330	0.1220	0.1050	0.1090
2	OOT1	Outgroup	0.1000	–	0.1000	0.1000	0.1290	0.1150	0.1200	0.1240	0.0960	0.1070
3	OIF02	1	0.0980	0.0980	–	0.0180	0.0550	0.0510	0.0550	0.0490	0.0470	0.0580
4	OIF17	-	0.1000	0.0980	0.0280	–	0.0510	0.0440	0.0550	0.0450	0.0470	0.0580
5	OIF19	2a	0.1040	0.1180	0.0470	0.0460	–	0.0550	0.0690	0.0670	0.0690	0.0760
6	OIF25	2b	0.1050	0.1080	0.0430	0.0410	0.0440	–	0.0600	0.0620	0.0730	0.0730
7	OIF03	3a	0.1160	0.1120	0.0570	0.0570	0.0700	0.0620	–	0.0310	0.0710	0.0750
8	OIF13	3b	0.1080	0.1100	0.0480	0.0470	0.0630	0.0560	0.0280	–	0.0670	0.0710
9	OIF06	4	0.0960	0.0980	0.0500	0.0520	0.0640	0.0620	0.0690	0.0650	–	0.0360
10	OIF24	–	0.0950	0.1020	0.0520	0.0540	0.0650	0.0630	0.0730	0.0660	0.0330	–

Table 4 Average within-group and range of uncorrected genetic distances for the clades identified in Fig. 2.

Clade	Average within-group genetic distance	Range of genetic distances
Clade 1	0.005	0.000–0.014
Clade 2a	0.018	0.000–0.026
Clade 2b	0.001	0.000–0.002
Clade 3a	0.000	0.000
Clade 3b	0.001	0.001
Clade 4	0.002	0.000–0.005

analyses determined that both the Chesterfield and Wanganui populations lie outside of the main *O. infrapunctatum* group, topology tests of this hypothesis were not applicable and were therefore not completed.

DISCUSSION

Hardy (1977) found little genetic distance among populations in his electrophoretic analysis of haemoglobin proteins in *O. infrapunctatum*, and proposed that intraspecific diversity within *O. infrapunctatum* arose during the last glacial period. He proposed that the species was isolated in a glacial refugium in Nelson/Marlborough and subsequently expanded northward through the central North Island and southward to southern Nelson. Populations from Westland were undiscovered at that time, but presumably under this scenario would have arisen by southward migration during the current interglacial period from the postulated Nelson/Marlborough refugium. However, in this study we present evidence for pre-Pleistocene divergence of major lineages within *O. infrapunctatum* and suggest that separate glacial cycles acted on this pre-existing diversity to create contrasting patterns of genetic structure within this species.

Genetic divergences among lineages within *O. infrapunctatum* are extremely deep. For example the uncorrected genetic distance between populations from Hokitika (OIF19; Clade 2) and Chesterfield (OIF3; Clade 3) is 6.9%, although these populations lie within 15 km of each other. The maximum genetic distance between lineages within *O. infrapunctatum* is 7.6%, between populations from Westport (OIF24) and Hokitika (OIF19), towns in Westland that lie 140 km apart. Our calibration for the evolutionary rate of *ND2*, of 1.4% Myr^{-1}, places this divergence before the Pleistocene, at 5.4 Ma. The depth of divergence among lineages we found within *O. infrapunctatum* shows clearly that, contrary to Hardy's (1977) hypothesis, much of the genetic diversity within this species was already present before the onset of glacial cycles in New Zealand. Molecular studies increasingly implicate Miocene and/or Pliocene processes as causal factors in adaptive radiations and allopatric divergence in many New Zealand taxa, including invertebrates (Buckley *et al.*, 2001; Trewick,

Table 5 Results of Shimodaira & Hasegawa (1999) tests of alternative topologies regarding the taxonomic status of divergent populations within *Oligosoma infrapunctatum*.

Alternative topology	–ln *L*	Difference in –ln *L*	*P*-value
Optimal tree	6377.13621		
'Paparoa Skink' lies outside *O. infrapunctatum*	6486.26863	109.13242	0.000
'Denniston Skink' lies outside *O. infrapunctatum*	6479.74274	102.60653	0.000
'Chesterfield Skink' lies outside *O. infrapunctatum*	(supported by optimal tree)	–	–
South North Island population lies outside *O. infrapunctatum*	(supported by optimal tree)	–	–

A significant *P*-value (< 0.05) indicates that the alternative topology is significantly different from the maximum likelihood (ML) tree.

2001; Chinn & Gemmell, 2004; Trewick & Morgan-Richards, 2005; Apte *et al.*, 2007; Haase *et al.*, 2007), galaxiid fish (Waters *et al.*, 2001) and skinks (Berry & Gleeson, 2005; Greaves *et al.*, 2007; Hare *et al.*, 2008).

The deep genetic divergences we found within *O. infrapunctatum* also have taxonomic implications, suggesting that *O. infrapunctatum* is actually a complex of cryptic species, which diverged in the Pliocene. In particular, our phylogenetic analyses and topology testing support the distinctiveness of *O. infrapunctatum* 'Chesterfield' (Clade 3a), contrary to previous allozyme work (Miller, 1999). Similarly, we found genetic support for the distinctiveness of *O. infrapunctatum* 'Southern North Island' (Clade 4). However, our genetic data and topology testing did not support the distinctiveness of *Oligosoma* sp. 'Paparoa' and *Oligosoma* sp. 'Denniston', which fell within the main *O. infrapunctatum* group (Clade 1). Morphological work will be needed to determine the taxonomic status of genetically distinct *O. infrapunctatum* populations, with the description of new species where appropriate.

Oligosoma infrapunctatum is of conservation interest, classified by New Zealand's Department of Conservation (DOC) as a threatened species in gradual decline due to human impact (Hitchmough *et al.*, 2007). Internationally, it is recorded on the IUCN 'Red List' as Lower Risk: Near Threatened (Hilton-Taylor, 2000). Factors thought to increase its vulnerability include its large body size, which decreases its ability to recover from predation (Whitaker, 1978), and its patchy distribution (Whitaker & Gaze, 1999; Whitaker & Lyall, 2004). Although widespread, *O. infrapunctatum* occurs in small, localized populations, often on unprotected land that is especially vulnerable to agricultural or exotic horticultural modification (Whitaker & Gaze, 1999; Whitaker & Lyall, 2004). It is not known whether its patchy distribution pattern is natural or human-induced, but this pattern increases the species' vulnerability to stochastic population loss (Whitaker & Lyall, 2004). Our study has revealed several deeply divergent clades and lineages within *O. infrapunctatum*, most of which have representatives in Westland and north-west Nelson. We note therefore that effective management of *O. infrapunctatum* hinges on the West Coast and Nelson/Marlborough Conservancies because these regions safeguard most of the genetic variation and structure that exists within this species.

Given that major lineages of *O. infrapunctatum*, which potentially constitute new species, diverged before the Pleistocene, is there evidence that glacial cycles have affected the distribution of this species? Phylogeographical patterns within *O. infrapunctatum* suggest that glacial cycles have in fact modified patterns of genetic diversity within *O. infrapunctatum* that first arose during the Pliocene. The pattern in Clade 1 of our phylogeny, characterized by shallow divergences and allopatric lineages, is evidence that populations have been in contact until recently but that recent gene flow has been low enough to permit genetic divergence (a 'Category III' pattern as described in Avise, 2000). Such a pattern could arise, for example, by recent expansion from glacial refugia, the scenario proposed for *O. infrapunctatum* by Hardy (1977). The shallow divergences among Clade 1 haplotypes in *O. infrapunctatum*, to a maximum of 1.4%, may indeed place this radiation within the Pleistocene (1.0 Ma). A Category III phylogeographical pattern in freshwater snails (*Potamopyrgus antipodarum*) in the South Island of New Zealand, has been interpreted as evidence of the confinement of ancestral haplotypes within glacial refugia and genetic isolation of populations following post-glacial expansion (Neimann & Lively, 2004). A phylogeographical study of tree-dwelling bats, *Mystacina tuberculata*, likewise suggested rapid expansion southward through Westland from a Nelson refugium, during the current interglacial (Lloyd, 2003). Based on the phylogeographical pattern present within Clade 1 of *O. infrapunctatum*, and the low levels of genetic differentiation within it, we suggest that this clade may have arisen, as postulated by Hardy (1977), by post-glacial expansion from refugia in the north of the South Island.

A contrasting phylogeographical pattern is seen in Clades 2, 3 and 4, characterized by deeply divergent allopatric lineages, providing evidence of long-standing barriers to gene flow (a 'Category I' pattern as described in Avise, 2000). However, this pattern can also arise by extinction of intermediate lineages (Avise, 2000). It is unlikely that extrinsic geographical barriers have caused this pattern in *O. infrapunctatum*, since the phylogeographical pattern within Clade 1 of the same species shows that the Westland coastal corridor does not contain long-standing barriers to the dispersal of *O. infrapunctatum*. The pattern is therefore more likely to have arisen by the extinction of most of the ancestral haplotypes within Clades 2, 3 and 4, leaving the few remaining lineages revealed in our modern phylogeny. However, this implies that lineage sorting has acted differently on Clades 2, 3 and 4 than on Clade 1, where a radiation of closely related haplotypes remains. We suggest therefore that these contrasting phylogeographical patterns within *O. infrapunctatum* are the result of post-glacial expansion during different interglacial periods. Under this scenario, Clade 2, 3 and 4 lineages expanded out of a 'mosaic of refugia' (Trewick, 2001) and through Westland during past interglacials, creating Category III patterns (shallow divergences, allopatric haplotypes) like that seen in Clade 1 now. Extinction of intermediate haplotypes by repeated cycles of glacial climate change, and accelerated drift in small populations, left only a few deeply divergent lineages persisting by chance in isolated locations in Westland. Interestingly, this would require the *in situ* persistence of populations of *O. infrapunctatum* in Westland throughout multiple Pleistocene glacial cycles. This is surprising but seems plausible, given that although individual glaciers reached the Tasman Sea from the Southern Alps, unglaciated areas in Westland are thought to have been protected from the full force of glacial climate change, because of the high rainfall that occurs west of the Alps (Suggate, 1990).

We also found evidence in Clade 1 for recent gene flow from the South Island northward into Cook Strait, supporting the existence of a recent land connection between the South Island and Cook Strait islands. Clade 1 occurs only in the South Island, except in two instances, firstly on Stephens Island in Cook Strait (OIF01, OIF10) and secondly in the Mount Bruce wildlife refuge in the south of the North Island (OIF21). The *ND2* haplotype discovered in the Mount Bruce sample is identical to the haplotype found on Stephens Island, the only instance in our study of an identical haplotype shared between two locations. We therefore suggest that this is probably the result of human translocation of a skink from Stephens Island to the wildlife refuge. We did not find any other instances of closely related haplotypes on both sides of Cook Strait, and we did not therefore find evidence of recent, Pleistocene gene flow between the North and South Islands as predicted by Hardy's (1977) northward expansion hypothesis for *O. infrapunctatum*. This adds to a growing body of evidence from molecular studies on brown kiwi (*Apteryx australis*), cicadas (*Maoricicada campbelli*), bats (*M. tuberculata*), land snails (*Wainuia umula*) and skinks (*Oligosoma lineoocellatum*), which have dated divergences between North and South Island clades to the Pliocene and early Pleistocene (Baker *et al.*, 1995; Buckley *et al.*, 2001; Efford *et al.*, 2002; Lloyd, 2003; Greaves *et al.*, 2007). These studies support the contention that if glacial land bridges existed across Cook Strait during the late Pleistocene, they were not sufficiently long-lived to provide suitable routes for the migration of terrestrial species (Lewis *et al.*, 1994; Worthy & Holdaway, 2002).

The genetic legacy of multiple glacial cycles is evident in the contrasting patterns of genetic diversity we have uncovered within *O. infrapunctatum*. The processes that originally drove the genetic divergence of lineages within *O. infrapunctatum* are likely, as in many other New Zealand taxa, to have been pre-Pleistocene. The geological and climatic history of New Zealand is complex and, as in Northern Hemisphere systems, it is increasingly evident that a wide range of historical processes, rather than simply those consequent upon glacial cycles, have driven intraspecific diversity within many taxa.

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