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ScienceDirect
Molecular Phylogenetics and Evolution 45 (2007) 729–739
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Short Communication

Phylogeography of the spotted skink (*Oligosoma lineoocellatum*) and green skink (*O. chloronoton*) species complex (Lacertilia: Scincidae) in New Zealand reveals pre-Pleistocene divergence

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Received 19 January 2007; revised 5 June 2007; accepted 11 June 2007
Available online 20 June 2007

1. Introduction

The New Zealand skink fauna is diverse, comprising at least 28 species in two endemic genera, *Oligosoma* and *Cyclodina* (Daugherty et al., 1994; Gill and Whitaker, 2001). The taxonomy of New Zealand skinks has been problematic, complicated by cryptic species, overlapping morphological characters among species, and wide variation within many species across their ranges (Hardy, 1977). There is no comprehensive molecular phylogeny for New Zealand skinks, so many species remain undescribed, and the origin and evolutionary history of the fauna remain largely unknown. For example, the age of the entire New Zealand skink fauna has been variously dated to the Pliocene, the Miocene and the Oligocene (reviewed in Smith et al., 2007).

Oligosoma lineoocellatum (spotted skink) and *Oligosoma chloronoton* (green skink) form the most taxonomically difficult species complex of all New Zealand lizards (Whitaker et al., 2002). During its taxonomic history various populations have been split off from and then returned to the complex (McCann, 1955; Hardy, 1977). Hardy's (1977) taxonomic revision defined the modern species boundaries. In particular, Hardy (1977) formally recognised a second species long suspected to exist within the complex, naming it *Leiolopisma chloronoton* (Prior to 1995, *Oligosoma* skinks were part of the non-endemic genus *Leiolopisma*; Patterson and Daugherty, 1995). Genetic studies have been equivocal as to the status of *O. chloronoton*, with some supporting its distinctiveness (Hardy, 1977; Hay, 1998) but others finding no genetic differentiation from *O. lineoocellatum* (Towns et al., 1985). The complex is thought to contain cryptic species (Whitaker and Gaze, 1999).

Oligosoma lineoocellatum and *O. chloronoton* show substantial intraspecific geographic variation in body size and morphology (Hardy, 1977). *Oligosoma lineoocellatum* reaches body sizes of up to 111 mm snout–vent length (SVL) (Hardy, 1977; Gill and Whitaker, 2001). Its distribution within the North Island is patchy and restricted (Fig. 1b); it occurs in just a few locations on the North Island, near Napier and in the Wellington region (Gill and Whitaker, 2001; Towns et al., 2002). *O. lineoocellatum* is also found on islands in Cook Strait, and in the Marlborough Sounds (Towns et al., 2002), and it is widespread on the South Island (Gill and Whitaker, 2001) (Fig. 1b). *Oligosoma chloronoton* reaches a maximum body size of 125 mm SVL (Whitaker et al., 2002), and is restricted to the southern South Island and surrounding islands (Towns et al., 2002; Whitaker et al., 2002) (Fig. 1b). Both *O. lineoocellatum* and *O. chloronoton* occur in coastal areas and through mid-altitudes up to the sub-alpine zone, to a maximum of 1700 m (Towns et al., 2002; Whitaker et al., 2002). They inhabit a wide range of habitats but prefer open shrubland, grassland or tussock habitat with stones, logs or vegetation for shelter (Towns et al., 2002; Whitaker et al., 2002). *O. lineoocellatum* may have been more widely distributed in the past, with Quaternary sub-fossils showing that it was once present in Otago, south of its current distribution limit (Worthy, 1997).

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1055-7903/$ - see front matter
doi:10.1016/j.ympev.2007.06.008

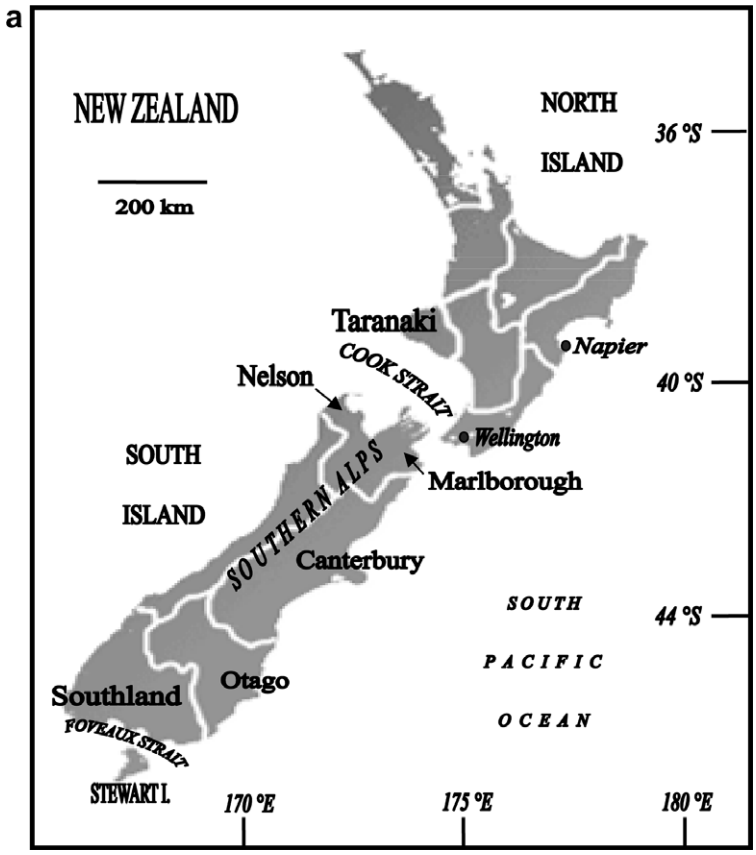

Fig. 1. (a) Map showing regions of New Zealand. (b) Map showing collection locations for *Oligosoma lineoocellatum* and *O. chloronoton* tissue samples listed in Table 1. The distributions of *O. lineoocellatum* (dashed line) and *O. chloronoton* (solid line) are shown (adapted from the BioWeb Herpetofauna (2006) database, New Zealand Department of Conservation). (c) Map showing the distribution of clades identified in Fig. 2a and b.

New Zealand's recent geological and climatic history has been complex (Cooper and Millener, 1993; Daugherty et al., 1993). Most of its mountains date from the Pliocene and are the result of a single, continuing cycle of tectonism which began in the Miocene (Gage, 1980; Suggate, 1982). From the late Pliocene, New Zealand experienced several glacial periods, marked by the formation of a continuous complex of extensive valley glaciers and ice fields along the Southern Alps (Suggate, 1990). Tectonic land movements and sea level changes during glacial cycles caused large-scale alterations to coastlines, including the formation of intermittent land bridges joining the two main islands of New Zealand (North Island and South Island) (Lewis et al., 1994). The relative impacts of these processes on biogeographic patterns in New Zealand taxa has long been a subject of debate. For example, ongoing debates include: (i) whether Pliocene tectonism was a more important factor than glacial climate change in shaping current distribution and diversity patterns (McGlone, 1985; Wardle, 1988; McGlone et al., 2001; Trewick and Wallis, 2001) and (ii) whether land bridges that formed during glacial periods allowed the interchange of terrestrial taxa between the North and South Islands (Lewis et al., 1994; Worthy and Holdaway, 2002).

It has been suggested that the current distributions of *O. lineoocellatum* and *O. chloronoton* and patterns of variation within the complex were shaped by Pleistocene glacial cycles, specifically migration across land bridges and range shifts into and out of glacial refugia (Hardy, 1977). Here we re-examine Hardy's (1977) hypothesis that Pleistocene processes suffice to explain distribution and genetic patterns within *O. lineoocellatum* and *O. chloronoton*, by using mtDNA sequence data (*ND2, ND4* and *cytb*) and a phylogeographic framework. We also examine taxonomic issues within the species complex, including the genetic distinctiveness of *O. lineoocellatum* and *O. chloronoton*, and the possibility of cryptic species.

2. Materials and methods

2.1. Sampling

Oligosoma lineoocellatum and *O. chloronoton* are classified as threatened species, and some populations are thought to be extinct, so we used existing specimen collections for our study. We obtained samples from the National Frozen Tissue Collection (NFTC; Victoria University of Wellington, New Zealand) and from ethanol-preserved museum specimens from Te Papa (National

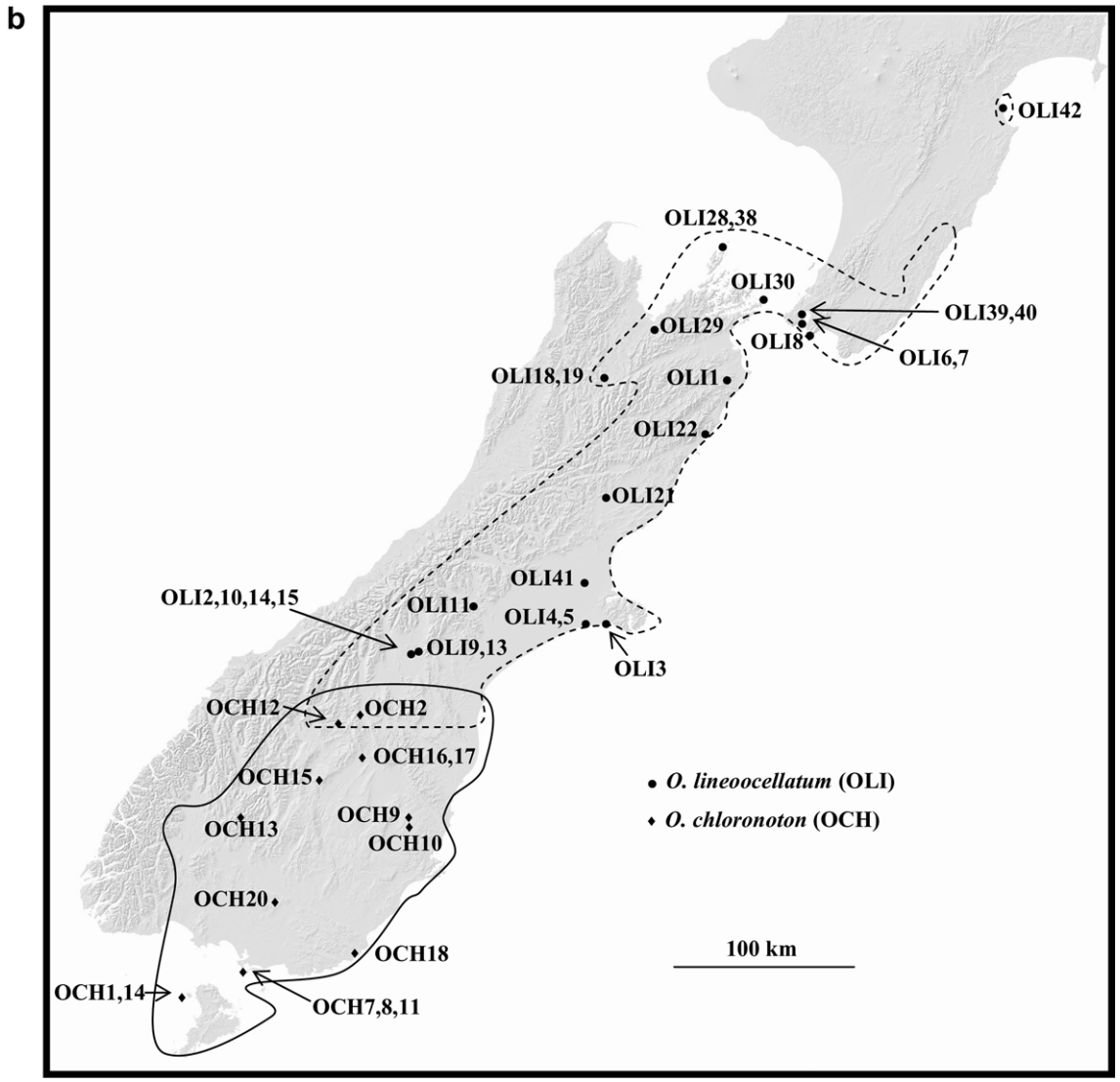

Fig. 1 (*continued*)

Museum of New Zealand, Wellington) for sites covering the entire known range of *O. lineoocellatum* and *O. chloronoton*. We also obtained some samples from private collections (Fig. 1b and Table 1). Two outgroup species, *O. otagense* (Otago skink) and *C. ornata* (ornate skink), were chosen based on a wider phylogenetic study of all endemic New Zealand skinks (Chapple, Daugherty and Ritchie, unpublished data).

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted using a standard phenol and chloroform protocol (Sambrook et al., 1989) followed by ethanol precipitation. The oligonucleotide primers listed in Table 2 were used with PCR 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 these fragments of the mitochondrial genome showed that they are sufficiently variable for intraspecific studies in squamate reptiles (Chapple and Keogh, 2004; Chapple et al., 2004, 2005; Keogh et al., 2005).

PCR was performed in 25 μL volumes containing 1 μL of DNA template, 1× reaction buffer (67 mM Tris–HCl (pH 8.8), 16 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$), 0.4 mg/mL BSA, 200 μM each dNTPs, 0.4 μM of each primer and 1 U of BioTherm DNA Polymerase (GeneCraft), or in 25 μL volumes containing 1 μL of DNA template, 1× reaction buffer (20 mM Tris–HCl (pH 8.4), 50 mM KCl), 1.5 mM $MgCl_2$, 0.4 mg/mL BSA, 200 μM each dNTPs, 0.4 μM of each primer and 1 U of Platinum *Taq* Polymerase (Invitrogen). All reactions were performed on an Eppendorf Mastercycler thermocycler. A typical PCR profile consisted of initial denaturation for 3 min at 94 °C, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 20 s at 55 °C and extension for 50 s at 72 °C, and a final extension step for 5 min at 72 °C. When amplification failed using this protocol, we used a gradient of annealing temperatures between 40 and 60 °C to obtain PCR product.

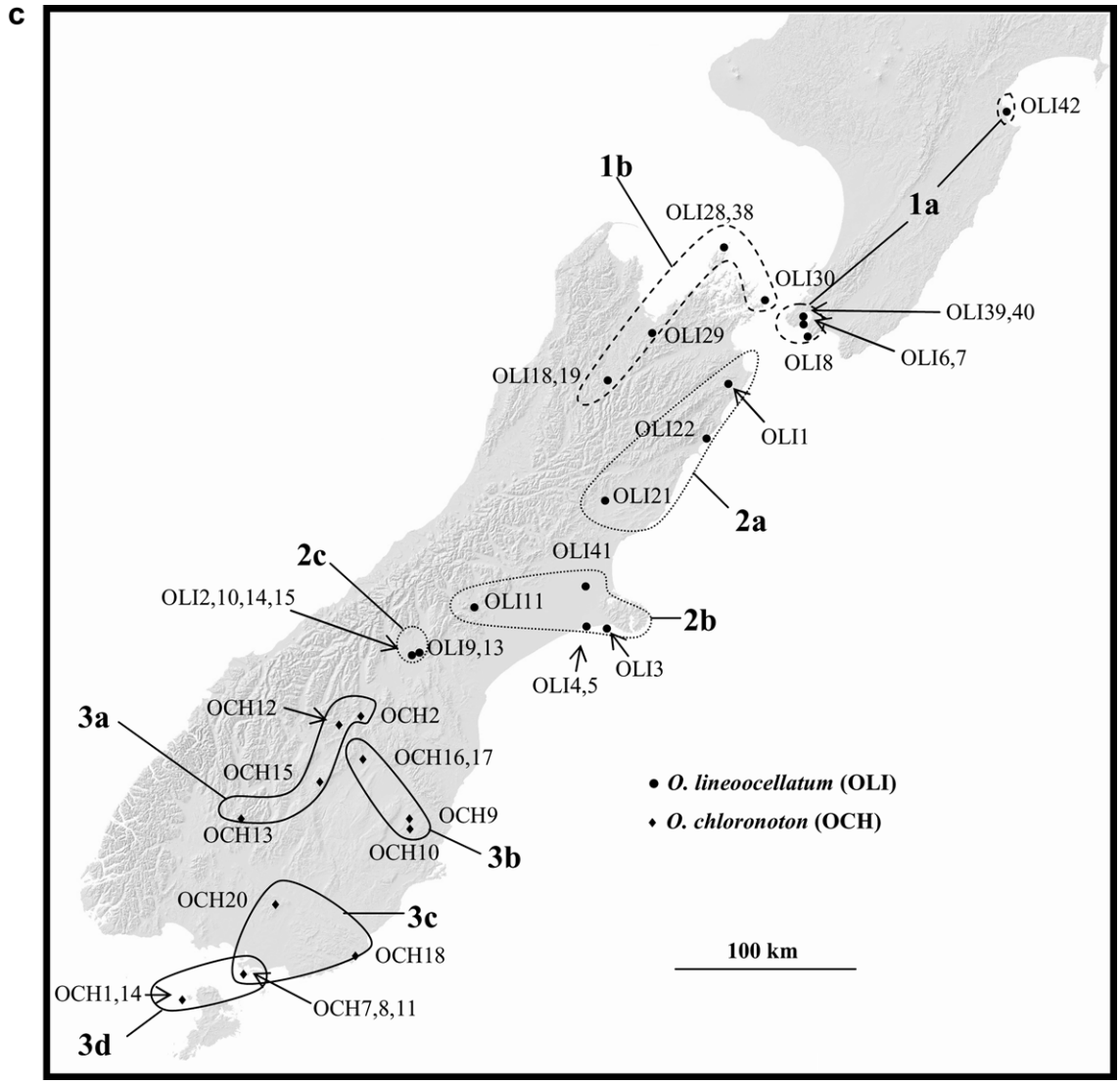

Fig. 1 (*continued*)

PCR products were purified using High Pure PCR Product Purification columns (Roche Diagnostics). The purified product was sequenced directly using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and then analysed on an ABI 3730 capillary sequencer.

2.3. Phylogenetic analyses

Sequence data were edited manually using ContigExpress in Vector NTI Advance 9.1.0 (Invitrogen). DNA sequences were aligned using ClustalX (Thompson et al., 1997) executed in MEGA 3.1 (Kumar et al., 2004). We translated all sequences to confirm that protein coding regions did not contain premature stop codons.

A partition-homogeneity test executed in PAUP* 4.0b10 (Swofford, 1998) confirmed that the phylogenetic signal from all three loci (*ND2*, *ND4* and *cytb*) was concordant (100 replicates; $P = 0.11$), and the sequences for each individual were therefore concatenated to create a single dataset which was used for subsequent phylogenetic analyses. A dataset for *ND2* only was also constructed. It contained *ND2* sequences from all of the individuals in the combined dataset, plus six additional sequences for individuals not included in that analysis and for which it was problematic to obtain PCR product, presumably because the museum specimens are several decades old (Table 1).

To determine the most appropriate model of evolution for the datasets, log-likelihood scores were generated using PAUP*, and used to conduct a hierarchical likelihood ratio test (hLRT) in ModelTest 3.7 (Posada and Crandall, 1998). The ModelTest analysis also provided estimates of base frequencies, substitution rates, the proportion of invariable sites, and the among-site substitution rate variation for each dataset.

The model and parameters obtained using ModelTest were used as settings in PAUP* to generate a maximum likelihood (ML) phylogenetic tree for the combined dataset and the *ND2*-only dataset. Heuristic searching was used, with the tree bisection-reconnection algorithm and random addition of sequences from the datasets. MrBayes 3.1.2

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

Sample	Species	Museum tissue code	Locality	Genbank Accession Nos.		
				ND2	*ND4*	*Cytb*
OCH1	*Oligosoma chloronoton*	FT555	Codfish Island	EF103955	EF103996	EF104031
OCH2	*O. chloronoton*	CD847	Tara Hill, Omarama	EF103956	EF103997	EF104032
OCH7	*O. chloronoton*	RE 5267	Tiwai Point	EF103957	EF103998	EF104033
OCH8	*O. chloronoton*	RE 5266	Tiwai Point	EF103958	EF103999	EF104034
OCH9	*O. chloronoton*	CD382	Emerald Creek	EF103959	EF104000	EF104035
OCH10	*O. chloronoton*	CD424	Macraes Flat	EF103960	EF104001	EF104036
OCH11	*O. chloronoton*	CD1280	Tiwai Point	EF103961	EF104002	EF104037
OCH12	*O. chloronoton*	CD1294	Lindis Pass	EF103962	EF104003	EF104038
OCH13	*O. chloronoton*	CD1904	Gorge Burn, Eyre Mountains	EF103963	EF104004	EF104039
OCH14	*O. chloronoton*	FT554	Codfish Island	EF103964	EF104005	EF104040
OCH15	*O. chloronoton*	FT584	Dunstan Mountains	EF103965	EF104006	EF104041
OCH16	*O. chloronoton*	FT593	Falls Dam	EF103966	EF104007	EF104042
OCH17	*O. chloronoton*	FT595	Falls Dam	EF103967	EF104008	EF104043
OCH18	*O. chloronoton*	FT3632	Catlins	EF103968	EF104009	EF104044
OCH20	*O. chloronoton*	CD2125	Hokonui Hills	EF103969	EF104010	EF104045
OLI1	*O. lineoocellatum*	FT302	Ward	EF103970	EF104011	EF104046
OLI2	*O. lineoocellatum*	FT3112	Tekapo	EF103971	EF104012	EF104047
OLI3	*O. lineoocellatum*		Kaitorete Spit	EF103972	EF104013	EF104048
OLI4	*O. lineoocellatum*		Birdlings Flat	EF103973	EF104014	EF104049
OLI5	*O. lineoocellatum*		Birdlings Flat	EF103974	EF104015	EF104050
OLI6	*O. lineoocellatum*	CD430	Ward Island	EF103975	EF104016	EF104051
OLI7	*O. lineoocellatum*	CD431	Ward Island	EF103976	EF104017	EF104052
OLI8	*O. lineoocellatum*	CD463	Cape Turakirae	EF103977	EF104018	EF104053
OLI9	*O. lineoocellatum*	CD1040	Northern Mt. Hay	EF103978	EF104019	EF104054
OLI10	*O. lineoocellatum*	CD1217	Tekapo	EF103979	EF104020	EF104055
OLI11	*O. lineoocellatum*	CD1064	Ashburton	EF103980	EF104021	EF104056
OLI13	*O. lineoocellatum*	FT2907	Mt. Hay	EF103981	EF104022	EF104057
OLI14	*O. lineoocellatum*	FT3211	Tekapo	EF103982	EF104023	EF104058
OLI15	*O. lineoocellatum*	FT3212	Tekapo	EF103983	EF104024	EF104059
OLI18	*O. lineoocellatum*		Lake Station	EF103984	EF104025	EF104060
OLI19	*O. lineoocellatum*		Lake Station	EF103985	EF104026	EF104061
OLI21	*O. lineoocellatum*	RE 4191 (S549)	Montrose Stream	EF103986	—	—
OLI22	*O. lineoocellatum*	RE 4192 (S550)	Waipapa Bay	EF103987	—	—
OLI28	*O. lineoocellatum*	CD601	Stephens Island	EF103988	—	—
OLI29	*O. lineoocellatum*	CD797	Aniseed Valley	EF103989	—	—
OLI30	*O. lineoocellatum*	FT239	North Brother Island	EF103990	—	—
OLI38	*O. lineoocellatum*	RE5217	Stephens Island	EF103991	—	—
OLI39	*O. lineoocellatum*	RE5262	Somes Island	EF103992	EF104027	EF104062
OLI40	*O. lineoocellatum*	RE5263	Somes Island	EF103993	EF104028	EF104063
OLI41	*O. lineoocellatum*		Orana Park, Christchurch	EF103994	EF104029	EF104064
OLI42	*O. lineoocellatum*	RE5320	Napier	EF447114	EF447112	EF447113
OOT1	*O. otagense*	CD1053	Central Otago	EF033053	EF033064	EF071065
COR1	*Cyclodina ornata*	FT188	Devonport, Auckland	EF103954	EF103995	EF104030

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

(Ronquist and Huelsenbeck, 2003) was used for Bayesian analysis of each dataset. To increase confidence that the analyses obtained a sampling of the full tree space rather than becoming trapped in local optima, a full analysis was run twice for each dataset. The analysis was run for 3,000,000 generations and was sampled every 100 generations. The program Tracer 1.3 (Rambaut and Drummond, 2003) was used to check for chain convergence. The first 25% of sampled trees was discarded as the burn-in phase, with the last 22,500 trees used to estimate the Bayesian posterior probabilities.

Bootstrap values and Bayesian posterior probabilities were used to assess branch support. The datasets were too large to do ML bootstraps, so neighbour-joining bootstrap analyses were performed for each dataset, using PAUP*. We used the distance corrections recommended by the ModelTest analyses, and ran 1000 bootstrap replicates for each dataset. Branches supported by bootstrap values of 70% or greater (Hillis and Bull, 1993) and/or posterior probability values greater than or equal to 0.95 (Wilcox et al., 2002) were considered to be supported by the data (Fig. 2a and b).

To estimate the time since the divergence of lineages within the *O. lineoocellatum* and *O. chloronoton* species complex, we calibrated the evolutionary rate of *ND2* by re-analysing data from Macey et al. (1998) for agamid

Table 2
Oligonucleotide primers used in this study

Mt region	Primer	5′–3′ sequence	5′ position	Source
ND2	L4437	AAGCTTTCGGGCCCATACC	3833	Macey et al. (1997)
	ND2F-infrapunctatum	GCATGATTYACCGGAAYATGAGACAT	4141	This study
	ND2R-infrapunctatum	GGGGCAAGKCCTAGTTTTATGG	4192	This study
	ND2r102	CAGCCTAGGTGGGCGATTG	4432	Sadlier et al. (2004)
ND4	ND4I	TGACTACCAAAAGCTCATGTAGAAGC	10,771	Forstner et al. (1995)
	ND4F-infrapunctatum	CCTCATAAACATAGCCCTCCCACC	11,217	This study
	ND4R-infrapunctatum	GGGGGATCAGTTAAAYAAYGAGGTG	11,274	This study
	ND4R-NZ	CCAAGRGTTTTGGTGCCTAAGACC	11,670	This study
	tRNA-Leu	TACTTTTACTTGGATTTGCACCA	11,691	Forstner et al. (1995)
Cytb	mtD25	CCATCCAACATCTCAGCATGATGAAA	14,940	Kocher et al. (1989)
	SkCytBR	TAGGCAAANARRAAGTAYCAYTCTGG	14,202	This study

Values in "5′ position" refer to the position of the 5′ base of the primer in the complete *Eumeces egregius* mtDNA sequence (Kumazawa and Nishida, 1999).

genus *Laudakia*. Specifically, we re-calculated 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 across each of the calibrated nodes from Macey et al. (1998) (1.5, 2.5 and 3.5 Mya), 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% per My (0.7% per lineage, per My) and is slightly faster than the rate of 1.3% per My found by Macey et al. (1998).

3. Results

The final combined dataset contained sequences from 35 individuals from 23 locations, as well as two outgroup sequences. For each individual, we obtained sequences from the mitochondrial loci *ND2* (550 bp), *ND4* plus *tRNA-His* and part of *tRNA-Ser* (773 bp), and *cytb* (610 bp). After concatenation, the aligned data set contained 1933 characters, of which 583 (30%) were variable, and 438 (23%) were parsimony-informative. For the ingroup only, the alignment contained 472 (24%) variable characters of which 399 (21%) were parsimony-informative. Base frequencies were unequal (A = 30.5%, T = 25.2%, C = 30.4%, G = 13.9%). A χ^2 test executed in PAUP* confirmed the homogeneity of base frequencies across all taxa in the dataset (df = 108, $P = 1.00$).

The *ND2*-only dataset contained sequences from 41 individuals from 28 locations, as well as two outgroup sequences. The aligned dataset contained 550 characters, of which 187 (34%) were variable and 144 (26%) parsimony-informative. For the ingroup only, 151 (27%) sites were variable, and 133 (24%) were parsimony-informative. Base frequencies were unequal (A = 31.3%, T = 22.6%, C = 32.1%, G = 14.0%). A χ^2 test executed in PAUP* confirmed the homogeneity of base frequencies across all taxa in the dataset (df = 126, $P = 1.00$).

The hLRT implemented using Modeltest selected the GTR + I + G substitution model as the most appropriate for the combined dataset ($-\ln L = 8271.0635$) and the TrN + G model as the most appropriate for the *ND2*-only dataset ($-\ln L = 2699.0759$). For both datasets, there was a strong bias towards transition substitutions (combined dataset rate matrix: A ↔ C = 5.40, A ↔ G = 119.78, A ↔ T = 3.35, C ↔ G = 4.42, C ↔ T = 49.12, G ↔ T = 1.00). For the combined dataset, Modeltest estimated the gamma shape parameter to be 2.4145 and the proportion of invariable sites to be 0.6159. For the *ND2*-only dataset, the gamma shape parameter was estimated to be 0.2343.

All phylogenetic analyses recovered three strongly-supported clades (Fig. 2a and b). Clades 1 and 2 represent *O. lineoocellatum*, while Clade 3 represents *O. chloronoton*. The average within-clade, uncorrected genetic distances for Clades 1, 2 and 3 were 2.1%, 3.9% and 5.5%, respectively. Average genetic distances between clades ranged from 8.3% (Clades 1 and 2) to 9.3% (Clades 1 and 3). The relationships among the three clades were not resolved. We identified nine regional subclades within the phylogeny: five subclades representing *O. lineoocellatum* and four subclades within *O. chloronoton* (Fig. 2a and b). Uncorrected genetic distances between representative members of different subclades ranged between 4.1 and 10.5% (Table 3). Average within-subclade genetic distances were between 0.06% and 1.4% (Table 4). The nine subclades were supported by high bootstrap values (100% in all cases) and posterior probabilities (1.00 in all cases) (Fig. 2a).

4. Discussion

4.1. Taxonomic implications

Morphological differences between northern populations classified as *O. lineoocellatum*, and southern populations classified as *O. chloronoton* have long been recognised. However, the taxonomic status of southern populations as a separate species has been debated. McCann (1955) included them within *L. lineoocellatum*, as *L. lineoocellatum* 'form parvicephallum'. However, using wider sampling, especially of *L. lineoocellatum* in the North

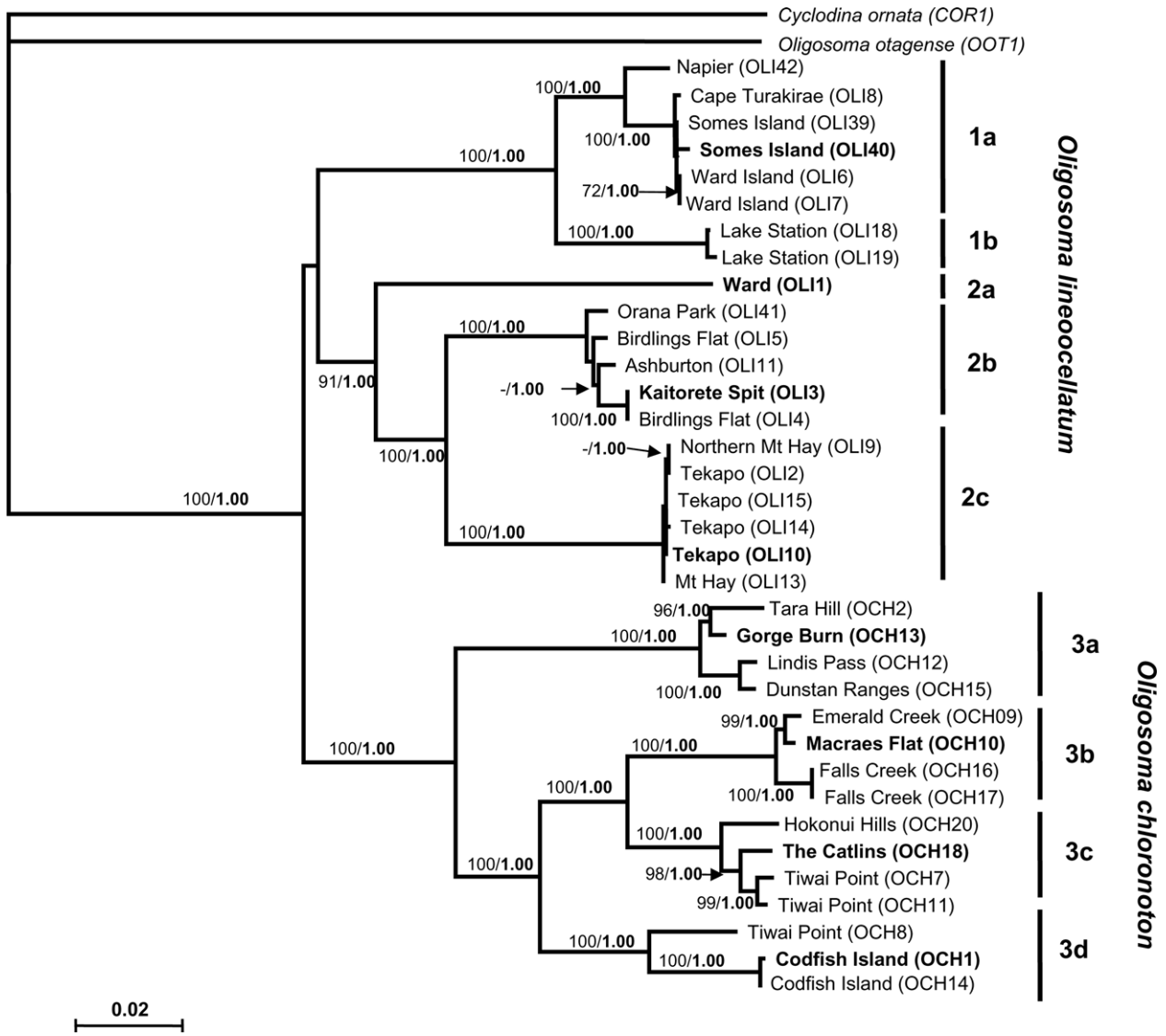

Fig. 2. (a) Maximum likelihood phylogram for *Oligosoma lineoocellatum* and *O. chloronoton*, based on the combined *ND2*, *ND4* and *cytb* dataset (1933 bp). Neighbour-joining bootstrap values are shown in plain text and Bayesian posterior probabilities in bold. Where no support value is shown, the node is unsupported, i.e., it has less than 70% bootstrap support and a Bayesian posterior probability less than 0.95. Three clades are identified within the species complex, comprised of five sub-clades within *O. lineoocellatum* and four sub-clades within *O. chloronoton*. (See Fig. 1c and Table 1). Representative lineages used to estimate genetic distances between clades are marked in bold. (b) Maximum likelihood phylogram for *Oligosoma lineoocellatum* and *O. chloronoton*, based on the *ND2* dataset (550 bp). Individuals not present in the combined dataset phylogram (a) are marked with an asterisk (*). Neighbour-joining bootstrap values are shown in plain text and Bayesian posterior probabilities in bold. Where no support value is shown, the node is unsupported, i.e., it has less than 70% bootstrap support and a Bayesian posterior probability less than 0.95. Three clades are identified within the species complex, comprised of five sub-clades within *O. lineoocellatum* and four sub-clades within *O. chloronoton* (see Fig. 1c and Table 1).

Island, Hardy (1977) recognised southern populations as a new species, which he named *L. chloronoton*. Genetic studies using electrophoresis of haem compounds (Hardy, 1977) and mitochondrial loci (*16S rRNA*; Hay, 1998) have also distinguished these species from one another, but because they used very limited sampling, especially within *O. chloronoton*, they are considered equivocal (Whitaker et al., 2002). However, our genetic data show *O. chloronoton* to be a strongly-supported monophyletic grouping, separated from the two major lineages of *O. lineoocellatum* by genetic distances over 9.0%. Our data therefore provide strong support for Hardy's (1977) hypothesis that *O. lineoocellatum* and *O. chloronoton* are separate species.

Morphological differences within *O. lineoocellatum*, between northern and southern populations were recognised by McCann (1955). He renamed Cook Strait populations as *Leiolopisma festivum*, based on larger body size and higher scale counts when compared with southern populations. Hardy (1977) examined additional specimens (McCann examined only one specimen from the North Island) and did not find sufficient differences among populations to justify this subdivision. He therefore returned *L. festivum* to synonymy with *L. lineoocellatum*. However,

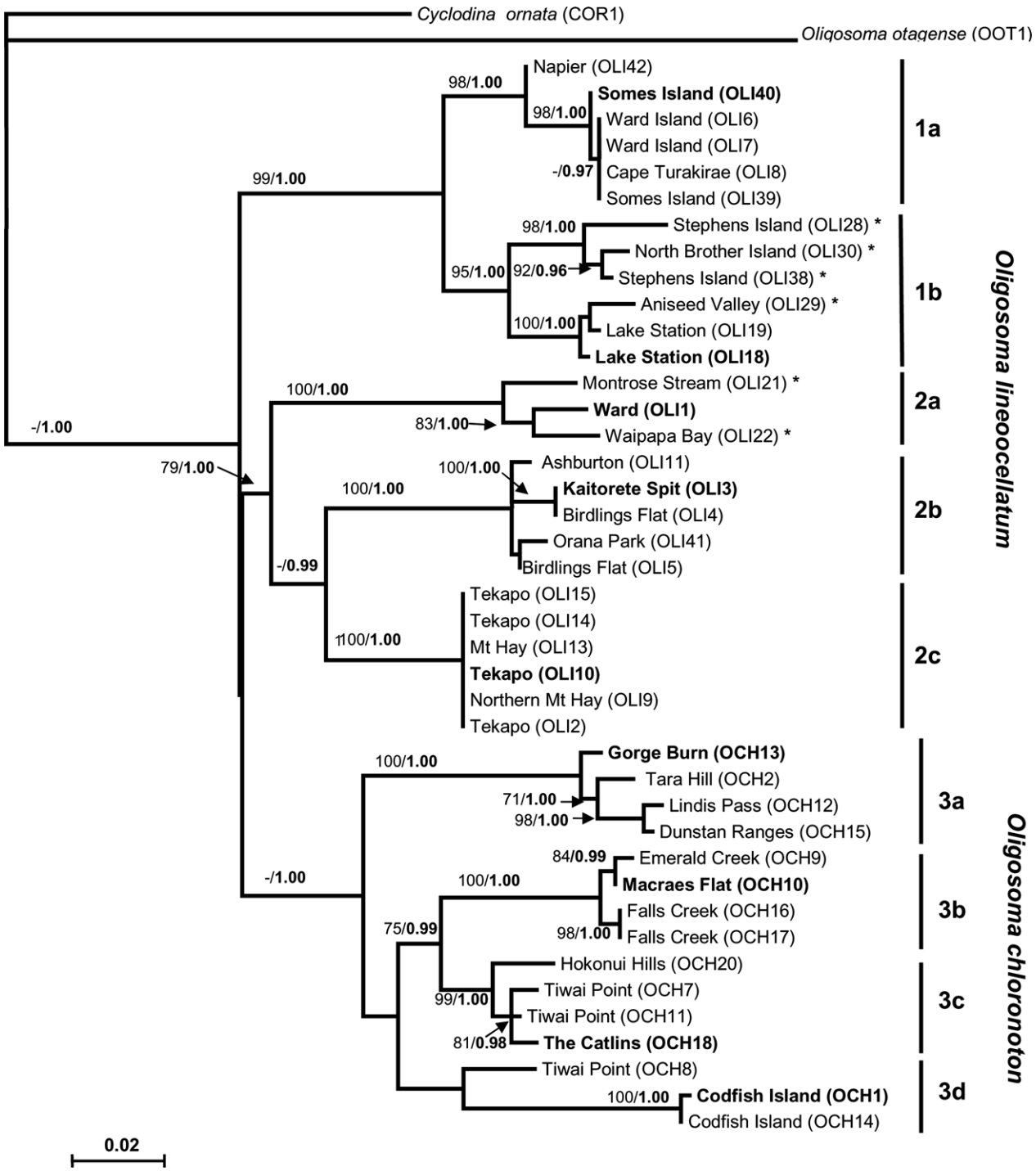

Fig. 2 (*continued*)

our phylogeny identifies a two major lineages within *O. lineoocellatum*, separated genetically by a distance of 8.3%, and geographically by the northern end of the Southern Alps. These lineages are as genetically distinct from one another as from *O. chloronoton*. Our data therefore provide support for McCann's (1955) hypothesis that *O. lineoocellatum* contains at least two species. However, our data support subdivision across the Southern Alps, rather than between Cook Strait populations and all other populations of *O. lineoocellatum*, as suggested by McCann (1955). Thus, our data suggest that further morphological work on this complex is warranted, with the description of new species where appropriate.

4.2. Phylogeographic implications

Hardy (1977) proposed a pivotal role for Pleistocene glacial cycles in shaping distributions and intraspecific patterns of variation within New Zealand endemic skink species. However, the genetic pattern revealed in *O. lineoo-*

Table 3
Uncorrected distance matrix for representatives (marked in bold) of each clade identified in Fig. 2a (complete dataset; below the diagonal) and Fig. 2b (*ND2*-only dataset; above the diagonal)

		Subclade	1	2	3	4	5	6	7	8	9	10	11
1	**COR1**	Outgroup	—	0.1400	0.1182	0.1182	0.1182	0.1182	0.1055	0.1073	0.1327	0.1291	0.1200
2	**OOT1**	Outgroup	0.1324	—	0.1309	0.1345	0.1600	0.1509	0.1400	0.1273	0.1491	0.1364	0.1255
3	**OLI40**	1a	0.1252	0.1211	—	0.0455	0.0982	0.0873	0.0836	0.0891	0.0836	0.0836	0.0764
4	**OLI18**	1b	0.1247	0.1169	0.0409	—	0.0982	0.0945	0.0891	0.0927	0.0855	0.0945	0.0855
5	**OLI01**	2a	0.1226	0.1267	0.0859	0.0890	—	0.0836	0.0709	0.0964	0.1018	0.0891	0.0927
6	**OLI03**	2b	0.1221	0.1283	0.0776	0.0828	0.0729	—	0.0655	0.0891	0.1000	0.0873	0.0855
7	**OLI10**	2c	0.1231	0.1298	0.0864	0.0874	0.0786	0.0564	—	0.0855	0.0964	0.0836	0.0891
8	**OCH13**	3a	0.1257	0.1185	0.0911	0.0921	0.0890	0.0781	0.0869	—	0.0745	0.0655	0.0800
9	**OCH10**	3b	0.1355	0.1330	0.0936	0.0942	0.0957	0.0864	0.0957	0.0729	—	0.0509	0.0691
10	**OCH18**	3c	0.1402	0.1340	0.0983	0.1045	0.0931	0.0874	0.0983	0.0698	0.0435	—	0.0691
11	**OCH01**	3d	0.1278	0.1185	0.0874	0.0905	0.0921	0.0843	0.0942	0.0714	0.0605	0.0616	—

Table 4
Ranges and averages of uncorrected genetic distances within the subclades identified in Fig. 2a

Clade	Genetic distance range	Average
1a	0.000–0.0171	0.0065
1b	0.0016	0.0016
2a	—	—
2b	0.000–0.0098	0.0072
2c	0.000–0.0010	0.0006
3a	0.0052–0.0197	0.0138
3b	0.000–0.0103	0.0072
3c	0.0041–0.0181	0.0129
3d	0.0005–0.0300	0.0202

cellatum and *O. chloronoton*, characterised by genetic distances of up to 10% among lineages, suggests that divergences among major lineages substantially pre-date the Pleistocene.

Within *O. lineoocellatum*, an uncorrected genetic distance of 9.8% (*ND2* only) is observed between representative lineages in Nelson and Marlborough, separated by the northern end of the Southern Alps. Our calibration for *ND2*, of 1.4% per million years, places this divergence within the Miocene, at 7 Mya. Likewise, there is 7.3% genetic distance between Marlborough and central Canterbury lineages (5.2 Mya; Miocene–Pliocene boundary), and 5.6% between central and southern Canterbury lineages (4 Mya; early Pliocene). The deepest genetic break within *O. chloronoton* occurs across the mountain ranges separating western and eastern Otago populations. The genetic distances between these lineages is 7.5%, which places their divergence at the Miocene–Pliocene boundary (5.4 Mya). East–west genetic breaks in Otago, though inferred to be of Pleistocene-age, have also been observed in the alpine weta, *Hemideina maori* (King et al., 2003), and in the subalpine grand skink, *Oligosoma grande* (Berry and Gleeson, 2005). Our results complement evidence from a wide range of taxa, which has found that Pliocene mountain uplift has been a causal factor in the divergence of several species of endemic insects (Buckley et al., 2001; Trewick, 2001; Trewick and Wallis, 2001; Chinn and Gemmell, 2004; Trewick and Morgan-Richards, 2005), galaxiid fish (Waters et al., 2001) and freshwater crayfish (Apte et al., 2007).

Shallower genetic divergences are observed across waterways that have intervened in the complex's distribution during New Zealand's recent history. During the Pliocene, the lower North Island and the South Island were joined by dry land, while a sea way separated them from the upper North Island (Lewis et al., 1994). The genetic distance between lineages from Napier and Wellington, areas which would have been separated by the Pliocene sea way, is only 1.3%, corresponding to divergence occurring 0.9 Mya, during the Pleistocene. The Pliocene sea way between Wellington and Taranaki is therefore probably not the cause of divergence between these lineages. It may be instead that lineages from the southern North Island colonised northward during the Pleistocene, when the sea way had receded.

The North and South Islands are now separated by Cook Strait, which first formed in the mid-Pleistocene (C. 0.45 Mya; Lewis et al., 1994). Dry land intermittently bridged Cook Strait during glacial periods, extending from the South Island to the Taranaki region, which is some 200 km north of Wellington (Lewis et al., 1994). The genetic distance between Nelson and Wellington populations, located on either side of Cook Strait, is 4.6%, corresponding to a divergence time of approximately 3.3 Mya. The depth of genetic divergence between populations on either side of Cook Strait and the current distribution of *O. lineoocellatum* in Wellington (the northern end of the Pliocene land bridge) rather than in Taranaki (the northern end of Pleistocene land bridges) suggests that migration occurred across the Pliocene land bridge between the modern-day North and South Islands, but not across late Pleistocene land bridges. Molecular studies on the brown kiwi (*Apteryx australis*; Baker et al., 1995), cicadas (*Maoricicada campbelli*; Buckley et al., 2001) and bats (*Mystacina tuberculata*; Lloyd, 2003) have dated divergences between North and South Island clades to C. 0.9 Mya, while a study of carnivorous land snail species (*Wainuia umula*), found genetic distances across Cook Strait that suggested separation for at least 4 My (Efford et al., 2002). Our results, taken together with these studies, support the idea 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.

Genetic structure across a waterway is also evident within *O. chloronoton*. Foveaux Strait, which intervenes in the distribution, forms a shallow sea strait between the South Island and Stewart Island and was bridged during glacial periods (Newnham et al., 1999). The latest opening of this sea way occurred only C. 11,500 years ago (McGlone and Wilson, 1996). However, there is 5.1% uncorrected genetic distance between the most closely-related haplotypes on either side of this waterway suggesting that the lineages had already diverged before the onset of glacial cycles. Tiwai Point on the South Island's south coast is notable for being the only location at which we found sympatric haplotypes. One of these mainland haplotypes (OCH8) was more closely related to Codfish Island haplotypes than to other mainland haplotypes. Sympatry of haplotypes in the grand skink, *O. grande*, was interpreted as evidence of admixture following expansion from glacial refugia (Berry and Gleeson, 2005). Since haplotypes within *O. lineoocellatum* and *O. chloronoton* are not sympatric elsewhere, we suggest that the most likely explanation for sympatry of these haplotypes is secondary contact, due to the migration of individuals across an historic Foveaux Strait land bridge.

Genetic distances between lineages within the complex reach over 10%, suggesting that the complex may be over 7 million years old. Given the depth of divergence within this species complex, it seems unlikely that the origin of the entire New Zealand skink fauna lies in the Pliocene as some studies have suggested (Robb, 1973; Towns, 1974; Bull and Whitaker, 1975; Hardy, 1977), or even within the last 7 My (Smith et al., 2007).

Acknowledgments

Matt McGlone and Phil Garnock-Jones provided comments that improved the manuscript. Benno Kappers provided access to the Department of Conservation's BioWeb Herpetofauna database. Marieke Lettink and Rod Hitchmough provided additional samples, and Robyn Howitt provided some of the extracted tissue samples used in this study. Lorraine Berry of the Allan Wilson Centre Genome Service did the sequencing for the study. We are grateful to Karen Britton for cataloguing and retrieving samples from the NFTC, and to Raymond Coory for assistance with access to samples from the Te Papa herpetology collection. Rod Hitchmough provided valuable comments during this study. This study was funded by grants from the Society for Research on Amphibians and Reptiles in New Zealand (SRARNZ) and the Victoria University of Wellington University Research Fund to P.A.R. and D.G.C., a grant from the Department of Conservation (Investigation No: 3662) to D.M.G and scholarships to S.N.J.G from Te Runanga a Iwi o Ngati Kahu, the Maori Education Trust and a VUW Graduate Award.

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