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Identification of a Rare Gecko from North Island New Zealand, and Genetic Assessment of Its Probable Origin: A Novel Mainland Conservation Priority?

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Abstract.—**The largest extant New Zealand gecko, *Hoplodactylus duvaucelii* (Duvaucel's Gecko), is a nocturnal, viviparous species of conservation concern. *Hoplodactylus duvaucelii,* once widespread throughout New Zealand, is now confined to offshore islands, the majority of which are free from all introduced mammalian predators (mice, rats, cats, mustelids, brushtail possums). A single *H. duvaucelii,* caught within a fenced reserve on North Island in 2010 was genotyped to determine whether it represents a recent introduction or a previously unknown native relict population. Genotypes from seven nuclear loci and a minimum spanning network of mtDNA haplotypes revealed two clusters representing southern (Cook Strait) and northern island populations. This genetic structure is concordant with variation between these two groups observed in body size, color pattern, and scalation. The mainland specimen was found to possess a mixture of morphological character states typical of northern and southern island populations. Although the individual possessed a unique mitochondrial haplotype, high heterozygosity, and a private nuclear allele, it was no more genetically distinct than conspecifics from isolated island populations. Comparisons with live captive geckos failed to provide evidence that the aberrant specimen represented a recent translocation. We infer that *H. duvaucelii* has survived naturally on North Island at very low population densities since the human-mediated introduction of novel predators 800 years ago. Our findings suggest a novel conservation priority, which should be prioritized for additional study in the immediate future.**

The largest extant species of gecko on New Zealand, *Hoplodactylus duvaucelii* (Duvaucel's Gecko), had a prehuman distribution that covered both North and South Islands of New Zealand (Worthy, 1987; Worthy and Holdaway, 2002; Fig. 1). *Hoplodactylus duvaucelii* is a large (snout–vent length up to 160 mm), nocturnal, viviparous, and particularly long-lived (>36 yr) species (Thompson et al., 1992; Cree, 1994).

Humans have facilitated the spread of numerous nonnative mammalian species such as rodents, dogs, cats, possums, hedgehogs, stoats, ferrets, and weasels in New Zealand. These introduced predators caused the extinction of many species of bird, at least two reptiles, one bat, and three species of frogs (Towns and Daugherty, 1994; Worthy and Holdaway, 2002). Many additional native species of birds, reptiles, amphibians, and insects now exhibit greatly reduced ranges as a result of the introduction of nonnative predators. Thus, today, many New Zealand endemic species are restricted to small offshore islands where mammalian predators currently are absent (Towns and Ballantine, 1993; Towns and Daugherty, 1994).

The introduction of New Zealand's nonnative predators occurred in two distinct waves. Maori settlers introduced kurı (domestic dogs: *Canis lupus familiaris*) and kiore (Pacific rats: *Rattus exulans*) in the 13th century (Wilmshurst and Higham, 2004). Although dogs have not been shown to have impacted native lizard populations substantially, rats clearly do threaten native gekkonid lizards like *H. duvaucelii*. This endemic has survived in the presence of kiore on several islands, but both abundance and variety of habitats used by *H. duvaucelii* are greatly reduced in the presence of this rodent (Whitaker, 1978; Hoare, 2006). As a result of the initial Maori-mediated introductions, *H. duvaucelii* populations likely were greatly reduced and limited to a subset of their original microhabitats. However, this species most likely still persisted in viable populations on the large islands of New Zealand when European visits, settlements, and a second wave of pest introductions began at the end of the 18th century.

Hoplodactylus duvaucelii populations usually do not persist in the presence of introduced ship rats (*Rattus rattus*), Norway rats (*Rattus norvegicus*), cats (*Felis catus*), mustelids (*Mustela erminea, Mustela furo, Mustela nivalis*), pigs (*Sus scrofa domesticus*), brushtail possums (*Trichosurus vulpecula*), and even house mice (*Mus musculus*, *M. domesticus*). *Hoplodactylus duvaucelii* disappeared from most islands where these species were introduced and where forests were cleared or were regenerating following human-induced fires (Towns and Daugherty, 1994). In contrast, surviving populations of *H. duvaucelii* occur, albeit at extremely low population densities, on Great Barrier and Little Barrier Islands, which have both areas of intact, diverse forest and very steep cliffs and bluffs that offer potential refuges from mammalian predators. Little Barrier Island formerly had cats and kiore. After removal of kiore from Little Barrier Island in 2004, *H. duvaucelii* was observed for the first time in several decades (Bellingham et al., 2010). This suggests the species' ability to persist at extremely low population levels when nonnative predators are present. On Great Barrier Island, which has all four rodent species, cats, dogs, pigs, and domestic livestock but no mustelids, possums, or hedgehogs, *H. duvaucelii* were reported intermittently until at least the 1970s, and one was found in 2011 in a rat trap in a sanctuary where intensive pest control is ongoing.

Because of their vulnerability to introduced predators, it has been assumed that *H. duvaucelii* was extinct on the larger islands of New Zealand (McCann, 1955). Data associated with museum

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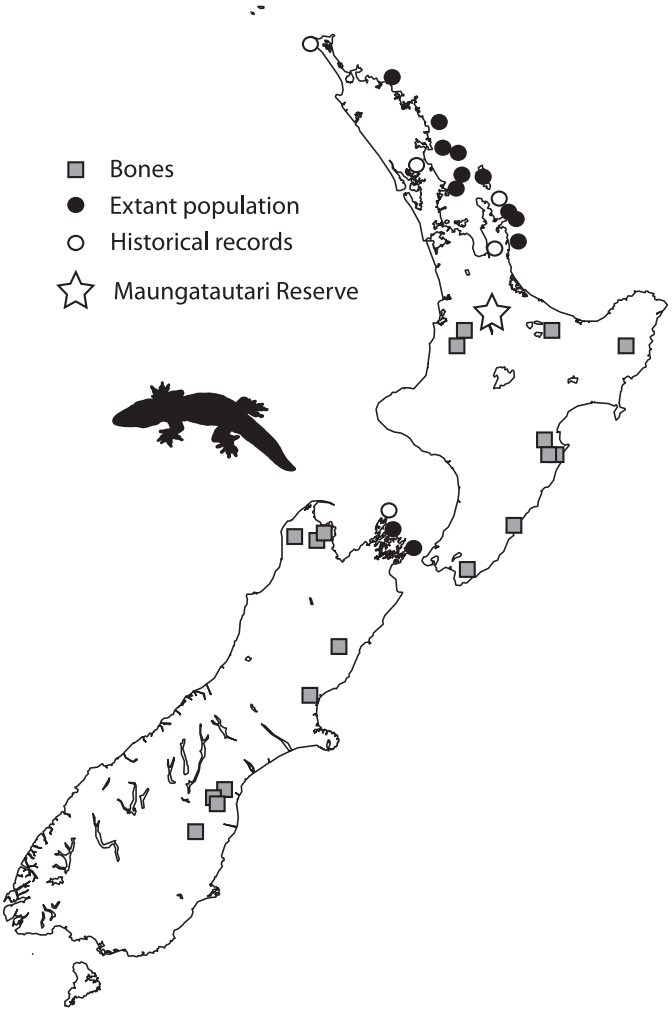

FIG. 1. The extensive prehuman distribution of the New Zealand gecko *Hoplodactylus duvaucelii*, inferred from bone deposits and museum records.

specimens of uncertain provenance suggest that this species was last collected from North Island at least 60 yr ago; *H. duvaucelii* is now restricted to small peripheral satellite islands off the coast of mainland New Zealand (Table S1, supplementary data). Although these island populations secure the species from extinction, there is considerable interest in restoring populations to former mainland range for purposes of ecological restoration.

When a distinctive adult male gekkonid lizard (Fig. 2) was found dead in a mouse trap within the Maungatautari Reserve (S 38°2.57′; E 175°33.25′) in March 2010, the occurrence raised the question as to its proper identification and, if *H. duvaucelii*, whether it could represent a native, possibly relic population. Alternatively we considered the possibility that this individual might have been translocated accidentally and released on the mainland from an unidentified peripheral satellite island population. Translocations of birds from New Zealand inshore islands to Maungatautari Reserve have involved species from islands where *H. duvaucelii* naturally occur (van Winkel et al., 2010; Parker, 2013). However, *H. duvaucelii* are uncommon on both of these islands, suggesting that accidental gecko translocations would be unlikely. Also, we considered a third possibility: that individuals of *H. duvaucelii* maintained in captivity might have been released in the Maungatautari Reserve. Despite the fact that private husbandry of *H. duvaucelii* requires official permission from national wildlife authorities (New Zealand Department of Conservation), many amateur enthusiasts maintain and breed *H. duvaucelii* in captivity.

We undertook the present phylogeographic study of *H. duvaucelii* expecting to detect similar geographic structure of genetic variation previously observed in the sympatric reptile *Sphenodon punctatus* (tuatara; Hay et al., 2009). Also, we strove to make use of phenotypic differences, which may reliably diagnose individuals from northern versus southern populations (RAH, pers. obs.). Individuals of *H. duvaucelii* from northern populations are considerably heavier (up to 118 g) than southern population (Cook Strait) adults (up to 49 g); southern population individuals possess generally brighter, boldly contrasting color patterns (RAH, pers. obs.). In addition, distinct scalation patterns diagnose specimens from particular geographic regions.

Our aims were first to confirm that the mainland specimen (Fig. 2) was correctly identified as *H. duvaucelii* and second to infer the most likely origin of the mainland specimen by collecting phylogeographic data using material from most extant *H. duvaucelii* populations, augmented with genetic material nondestructively sampled from live captive animals. Three alternative hypotheses of contrasting genetic patterns arise from the three possible sources of this individual: 1) direct accidental transfer from a peripheral satellite island during translocation of bird species; 2) unauthorized release of the individual from a captive population; and 3) identification of a natural mainland population, which has persisted without detection for more than 60 yr. We predicted that direct transfer would result in 1) shared haplotype(s) common to Korapuki (Mercury Island group) or Little Barrier Island (the known sources for bird introductions). New Zealand lizards are protected under the Wildlife Act 1953, but authority to legally hold *H. duvaucelii* in captivity can be granted by the New Zealand Department of Conservation (Trewick and Morgan-Richards, 2014). All captive animals are presumed to be the offspring of very few individuals. As a result, if the individual in question resulted from the release of a captive animal, it might be expected to 2) share a haplotype also found in captive individuals and be indistinguishable from a direct-transferred island individual. Alternatively, if the focal subject was the captive-produced offspring of parents from southern and northern islands, it would have distinctive allele combinations and relatively high heterozygosity, depending on population size and number of generations held at presumed bottleneck densities. Finally, a relictual natural population 3) might possess a unique mtDNA sequence or nuclear alleles not observed in other populations.

MATERIALS AND METHODS

To distinguish among these hypotheses, we obtained material from 30 *H. duvaucelii* samples collected from 17 different island populations and 16 samples from captive animals (Table 1). This included a genetic sample from a newly confirmed (January 2011) population on Great Barrier Island. No material was available from Little Barrier Island, but we were able to include samples from three islands in the Mercury group, including Korapuki. Finally, we included six samples nondestructively collected from the North Brother Island (collected as part of population translocation operations conducted by wildlife management authorities).

FIG. 2. The single *Hoplodactylus duvaucelii* specimen, captured in a mouse trap, in 2010 in the Maungatautari reserve, four years after eradication of most introduced mammalian predators. This is the first record of this species on mainland New Zealand in more than 60 yr.

Morphological Data.—We examined the specimen from Maungatautari (Te Papa Tongarewa, Museum of New Zealand [MONZ] catalog number RE.007381), focusing on traits of scalation because lower labial scales decrease gradually in size in individuals from northern populations but are abruptly smaller after the fourth scale in individuals from southern populations (RAH, pers. obs.).

Genetic Data.—DNA sequence data are available for all putative species of the known New Zealand gecko fauna (Chong 1999; Nielsen et al., 2011). Within Maungatautari Reserve three species of gecko have been observed: *Naultinus elegans*, *Dactylocnemis pacificus*, and *Mokopirirakau granulatus*. To confirm the identity of our unique specimen, and to allow inference of its possible origin, we genotyped five polymorphic microsatellite loci and obtained DNA sequence data from three polymorphic loci for 47 individuals of *H. duvaucelii* (Table 1). Genomic DNA was extracted from tissue samples (tail tips or toe clips) using a DNeasy tissue and blood kit (Qiagen, Valencia, CA, Inc., Valencia, CA). DNA was diluted to ~5 ng/μL for PCR reactions. Pairs of primers were used to amplify two mitochondrial fragments and seven nuclear loci for all specimens (Table 2). Polymerase chain reactions were performed in 20 μL or 10 μL volumes containing 200 μM dNTPs, 2.5 mM MgCl, 1 μM primers, 0.20 U of Eppendorf DNA polymerase, and 1–10 ng template DNA. Amplification cycles consisted of denaturation at 94°C for 60 sec followed by 35 cycles of 94°C for 20 sec, annealing between 50°C and 55°C for 15 sec, and 68°C for 90 sec. We sequenced four gene-fragments; 16S, ND2, RAG-1, and PDC (Table 2). Because of low primer concentration in the amplification reactions, product clean-ups were not required. Cycle sequencing used Perkin Elmer BigDye v3.1 chemistry following the manufacturer's protocols, with automated reading on an ABI3730, by the Massey Genome Service (http://genome.massey.ac.nz/), using one of the amplification primers. For each gene fragment, we sequenced in both directions a subsample of individuals that represented the diversity of alleles and haplotypes to confirm observed nucleotide substitutions, and remaining samples were sequenced in one direction only.

We used sequencher v4.9 (Gene Codes Corp., Ann Arbor, MI) to check, align and trim primer sequences from our DNA sequences, SE-AL v2.0 (Rambaut, 2002) to check for stop codons, and further examined our data in MacClade v4.0 (Madison and Madison, 2002). Genotypes of geckos that were heterozygous at either RAG-1 or PDC were resolved by determining which combination of alleles would result in the observed pattern of nucleotide heterozygosity. Given the low number of variable sites and only two alleles per locus, genotypes were unambiguous, eliminating the need to clone or use phasing software.

In MacClade, we concatenated 16S and ND2 fragments because these are inherited as part of a single unit (the mitochondrial genome). Sequences were condensed such that redundant taxa and characters were removed; this left only variable sites and one representative sequence for each distinct allele (or haplotype). Because of the simplicity of our data set, a minimum spanning network for mtDNA haplotypes was manually constructed without software using the principle of parsimony.

One of each microsatellite primer-pair was fluorescently labeled and amplified as described by Wong et al. (2011), and PCR products were sized using a capillary ABI3730 Genetic Analyzer and Geneious v6.1.6 (Drummond et al., 2011), equipped with Microsatellite Plugin v1.1 (Biomatters, Ltd., 2012). Evidence for long allelic drop-out and presence of null alleles were assessed with MICRO-CHECKER (Oosterhout et al., 2004), using a Bonferroni multiple comparisons corrections

TABLE 1. Genotypes of *H. duvaucelii* individuals used to determine the origin of an individual collected on mainland North Island New Zealand (coded Mnld; MONZ RE.007381). CD and FT codes as provided by National Frozen Tissue collection (Victoria University of Wellington). Putative location of origin for some captive animals was omitted as unreliable. Letter codes indicate unique mitochondrial haplotypes. Gaps indicate missing data. Microsatellite loci (Hduv03, Hduv20, Hduv06, Hduv05, Hduv04) genotypes are presented as allele lengths. Two alleles detected at each of two nuclear loci (Rag-1, PDC) were designated "A" and "B," respectively. H = number of loci at which the specimens are heterozygous.

Location				mtDNA																
Region	Island group	Island	Code	haplotype	Hduv03		Hduv20		Hduv06		Hduv05		Hduv04		RAG-1		PDC		H
Northern	Poor Knights	Aorangi Island	cd 1032	H	119	119	184	184	174	174	165	165	172	174	B	B	A	A	1
Northern	Hen and Chickens	Taranga Island	FT 576	T	122	122	194	184	174	176	165	165	178	178	B	B			2
Northern	Hen and Chickens	Taranga Island	FT 580	U	132	132	194	184	174	174	165	165	178	178	B	B	A	A	1
Northern	Hen and Chickens	Coppermine Island	FT 630	V	128	128	194	184	174	174	165	165	174	174	B	B	A	A	1
Northern	Great Barrier Island	Great Barrier Island	HdGB-260	J	119	119	192	192	174	174	161	165	176	174			A	A	1
Northern	Shoe/Slipper	Penguin	CD 984	W	122	119	188	198	174	174	165	165	176	174	B	B	A	A	3
Northern	Mercuries	Middle Island	FT 175	R	119	119	186	194	174	174	165	165	176	176	B	B			
Northern	Mercuries	Middle Island	FT 176	R	119	119	194	194	174	174	165	165			B	B	A	A	0
Northern	Mercuries	Green Island	FT 177	R	119	119	186	188	174	174	165	165	176	176	B	B	A	A	1
Northern	Mercuries	Green Island	Ft 178	R	119	119	186	186	174	174	167	165	176	178	B	B	A	A	2
Northern	Mercuries	Korapuki	FT 179	P	126	119	194	188	174	174	165	165			B	B	A	A	2
Northern	Alderman	Hernia Island	FT 560	M	124	122	188	188	174	174	167	167	176	174	B	B	B	B	2
Northern	Alderman	Middle Chain	FT 562	N	122	119	188	188	174	176	165	167	174	174	B	B	B	B	3
Northern	Alderman	Raumahuaiti	FT 564	L	122	119	188	194	174	174	167	167	176	174	B	B	A	B	4
Northern	Alderman	Raumahuanui	FT 566	O	119	119	188	188	174	174	167	167	174	174	B	B	A	B	1
Northern	Alderman	Hongiora	FT 567	K	122	119	188	188	176	176	169	167	176	176	B	B	B	B	2
Mainland	Maungatautari	Reserve	Mnld	S	124	122	188	188	174	176	159	167	182	182	A	B	B	B	4
Cook Strait	Brothers Island	North Brothers	cd 985	D	124	124	188	188	174	174	159	159	174	174	A	A	A	A	0
Cook Strait	Brothers Island	North Brothers	FT 2908	A	124	124	188	188	174	174	159	159	178	176	A	A	A	A	1
Cook Strait	Brothers Island	North Brothers	FT 2909	F	124	124	188	188	174	174	159	159	178	176	A	A	A	A	1
Cook Strait	Brothers Island	North Brothers	FT 277	C													A	A	
Cook Strait	Brothers Island	North Brothers	FT 278	A													A	A	
Cook Strait	Brothers Island	North Brothers	Hdb-254		124	124	188	188	174	174					A	A	A	A	0
Cook Strait	Brothers Island	North Brothers	Hdb-255	A	124	124	188	188	174	174	159	159	178	176	A	A	A	A	1
Cook Strait	Brothers Island	North Brothers	Hdb-256	A	124	124	188	188	174	174	159	159			A	A	A	A	0
Cook Strait	Brothers Island	North Brothers	Hdb-257	A							159	159	178	176	A	A	A	A	1
Cook Strait	Brothers Island	North Brothers	Hdb-258	A	124	124	188	188	174	174	159	159			A	A	A	A	0
Cook Strait	Brothers Island	North Brothers	Hdb-259		124	124	188	188	174	174	159	159	174	174	A	A	A	A	0
Cook Strait	Trios	Middle	FT 2046	G	130	130	190	194	174	174	159	159	178	176	A	A	A	A	2
Cook Strait	Trios	North	FT 2047		130	130	190	194	174	174	161	161	172	172	A	A	A	A	1
Cook Strait	Trios	South	FT 2048		130	130	190	190			159	159	178	176	A	A	A	A	1
Captive (RR)	1. Old female		Hdc-250	A	124	124	188	188	174	174	159	159	174	174	A	A	A	A	0
Captive (RR)	2. Male		Hdc-251	B	124	124	188	188	174	174	159	159	174	174	A	A			0
Captive (RR)	3. Male	Slipper Island	Hdc-252	R	124	119	194	186	174	174	165	165	180	176	B	B	A	B	4
Captive (RR)	4. Female	Slipper Island	Hdc-253		124	119	194	186	174	174					A	A	A	B	3
Captive (MK)	HD-12 Female 7.1	Coromadel island	Hdc-261	R	124	119	194	186	174	174	165	165			B	B	A	B	3
Captive (MK)	Hd-02	Slipper Island	Hdc-262	R	124	109			174	174	165	165			B	B	A	B	2
Captive (MK)	Hd-07 Male		Hdc-263	W	124	124			174	174					B	B	A	B	1
Captive (MK)	Cage 7.3	F1 HD12	Hdc-264	R	124	124			174	174							A	B	1
Captive (CK)	Female	F1 Penguin mother	Hdc-265	W	124	122	188	198	174	174	159	165	178	174	A	B	A	B	6
Captive (D)	Male G54	1980s × Mike Meads	Hdc-267	W	124	124	188	198	174	174			178	176	A	B	B	B	3
Captive (D)	Female G55	1980s	Hdc-268	W	124	122	188	198	174	174	159	165	176	174	A	B	A	B	6

TABLE 1. Continued.

Location				mtDNA													
Region	Island group	Island	Code	haplotype	Hduv03	Hduv20	Hduv06	Hduv05	Hduv04	RAG-1		PDC		H
Captive (D)	Male G226		Hdc-269	E	124	188	174				A	A	A	B	1
Captive (D)	Female G333		Hdc-270	B	124	188	174	159	167	178	A	A	A	A	1
Captive (D)	Female G802	2006, ex Bob Parker	Hdc-272	A	124	188	174	159	159		A	A	A	A	0
Captive (D)	Male G1096	2006, ex Bob Parker	Hdc-273	I	126	186	190	174	174	165	165	176	174	B	B	A	A	3
Captive (D)	Male G957	2006, ex Bob Parker	Hdc-274	B	124	188	194	174	174	159	165	176	176	A	B	A	A	4
Number of alleles/haplotypes				24	8	8	2	5	6	2		2					

approach and employing a 95% confidence interval and 10,000 repetitions. Evidence of isolation-by-distance was sought using a mantel test of the correlation of pairwise linear geographic distances and pairwise F_{ST} (or pairwise *P*-distance for mtDNA) with 1,000 permutations in GENEPOP 4.0.10. The seven nuclear loci were combined, and all wild-sampled genetic samples were included in the analysis. One haplotype per island was included for mtDNA data to represent the two islands where multiple haplotypes were sampled (Taranga and North Brothers Islands). The pairwise F_{ST}-matrix was estimated from the multilocus genotypes (seven loci) of geckos from 18 natural populations and used to infer a tree using the NeighborNet algorithm (Bryant and Moulton, 2004) implemented in Splitstree v.4.13.1 (Huson and Bryant, 2006).

Population structure was assessed without a priori group assignments using a Bayesian approach implemented by STRUCTURE v2.3.4 (Pritchard et al., 2000) and excluding samples from captive animals. We ran STRUCTURE analyses using an admixture model with independent allele frequencies, 100,000 generations designated as burn-in, followed by 100,000 Markov chain Monte Carlo randomizations, and the number of groups (K) variably set from 1 to 6 with five iterations of each run. The optimum value of K was found using the ΔK method except for K = 1, which was determined by examination of the bar-plots (Evanno et al., 2005). To combine five iterations for visualization, STRUCTURE output was converted via Structure Harvester Web v0.6.93, CLUMPP V1.1.2 (Jakobsson and Rosenberg, 2007) and DISTRUCT v1.1 (Rosenberg, 2004).

RESULTS

Morphology.—The specimen (MONZ RE.007381) from Maungatautari, North Island, New Zealand, was identified as an adult male using external sexual characteristics (presence of actively secreting precloacal organs/pores, well-developed cloacal spurs, and hemipenal bulges). The unique specimen was identified as *H. duvaucelii* on the basis of its size (snout–vent length 114 mm, weight 37 g) and digital morphology. The specimen's small size is typical of adults from southern (Cook Strait) island populations (Jewell and Morris 2008). The color and pattern of the Maungatautari specimen partially resembled (Fig. 2) individuals from Cook Strait islands. In contrast, the infralabials decrease gradually, as is typical in northern island populations.

Mitochondrial DNA.—We sequenced and aligned 800bp of 16S and 1,100bp of ND2. Sequences from three *H. duvaucelii* from a previous study were included (Nielson et al., 2011; HM9 = FT 174 [GU459843 GU460041]; HM10 = FT 277 [GU459844 GU460042]; HM11 = FT 278 [GU459845 GU460043]). Nineteen haplotypes were identified from 24 individuals with a maximum divergence between any two pairs of haplotypes reaching a maximum of 65 steps (or 3.4%). A minimum-spanning network resolved two clusters separated by 43 nucleotide substitutions and corresponding to two geographical regions: 1) southern (Cook Strait) populations and 2) northern islands and Maungatautari. Within the northern islands, individuals from Aorangi Island (Poor Knights group) and Great Barrier Island possessed haplotypes that differ by at least 16 nucleotide substitutions from all other northern haplotypes (Fig. 3). The Maungatautari individual had a unique mitochondrial haplotype but differed from haplotypes found on the Shoe and Slipper Islands and Hen and Chicken Islands by only two mutational steps.

Nuclear Loci.—DNA sequences of RAG-1 (810bp) resolved two alleles that differ by four nucleotide substitutions. One was

TABLE 2. The primers used to amplify DNA from seven nuclear loci and two mitochondrial fragments to infer probable origins of gecko found in Maungatautari reserve New Zealand.

Loci	Primer name	Primer sequence (if designed for this study) or reference
Mitochondrial ribosomal 16S gene fragment	16Sc	Reeder, 1995
Mitochondrial ribosomal 16S gene fragment	H3056	Chapple and Keogh, 2004
Mitochondrial subunit 2 NADH dehydrogenase	ND2(Hduv)Fab	5′-CTCCCAGAAACRCTACAAGG-3′
Mitochondrial subunit 2 NADH dehydrogenase	COIR8	Weisrock et al., 2001
Nuclear Recombination activating gene-1	RAG-1(Hduv)F:	5′-GACAAAGTCAGGGAGGGACA-3′
Nuclear Recombination activating gene-1	RAG-1(Hduv)R	5′-TCCCAGAGCAATTTCTTCGT-3′
Nuclear Phosducin	PDC(hduv)F	5′-AAAACGAAACTTGCCTACGG-3′
Nuclear Phosducin	PDC(Hduv)R	5′-ACTTACGAGCTCCCCACCTC-3′
Nuclear microsatellite Hduv03	Hduv03F	Wong et al., 2011
Nuclear microsatellite Hduv03	Hduv03R	Wong et al., 2011
Nuclear microsatellite Hduv04	Hduv04F	Wong et al., 2011
Nuclear microsatellite Hduv04	Hduv04R	Wong et al., 2011
Nuclear microsatellite Hduv05	Hduv05F	Wong et al., 2011
Nuclear microsatellite Hduv05	Hduv05R	Wong et al., 2011
Nuclear microsatellite Hduv06	Hduv06F	Wong et al., 2011
Nuclear microsatellite Hduv06	Hduv06R	Wong et al., 2011
Nuclear microsatellite Hduv20	Hduv20F	Wong et al., 2011
Nuclear microsatellite Hduv20	Hduv20R	Wong et al., 2011

restricted to northern populations and the other to southern populations (both were detected in the sample of captive animals). The individual from Maungatautari was heterozygous for these two RAG-1 alleles. DNA sequences of PDC (358bp) resolved two alleles that differed by a single nucleotide substitution. The specimen from Maungatautari was homozygous for an allele otherwise found only in the Alderman Island group (also detected in captive specimens; Table 1).

For our five polymorphic microsatellite loci, no evidence of long allele drop-out or null alleles was detected. The individual from Maungatautari was heterozygous at three of these loci and at locus Hduv04 was homozygous for a private allele. Many alleles were found in more than one island group, although differentiation among regions (southern and northern populations) was evident in unique alleles. The genotype network was concordant with the inferences from mtDNA haplotypes (Fig. 3), with clusters of genotypes corresponding to northern and southern populations. The Maungatautari specimen clusters with geckos from the Alderman Island chain, and although unique it is not more distinct than other isolated gecko genotypes. Correlations between linear geographic distance and genetic distance (seven nuclear loci combined [F_{ST}] and mtDNA *P*-distances) were positive and provided evidence of isolation by distance within our sample ($P > 0.0001$ for both measures of genetic distance).

Captive offspring (F_1, F_2, or backcross) from parental northern and southern islands exhibited the highest heterozygosity (Fig. 4). The Maungatautari specimen possessed higher heterozygosity than most wild individuals, consistent with the hypotheses of having originated from a large population.

The optimal number of groups inferred from STRUCTURE analyses was K = 2 (Fig. 5A). Northern island geckos clustered together, and Brothers Island samples grouped with Trio Islands (southern islands; with some apparent admixture; Fig. 5). This analysis suggests that the individual from Maungatautari represents a 20–22% southern, 80–78% northern genetic amalgamation. Under K = 3, a third group formed, consisting of Alderman and Maungatautari samples (Fig 5B).

Inferred ancestry for the 16 captive specimens when K = 2 (Table S2, supplementary data) indicated that a total of nine individuals were pure southern or northern ancestry (>0.950), whereas seven others were clearly F_1s or backcrosses. These individuals of mixed parentage had variable but generally intermediate expression of the difference in scale size between infralabial 4 and 5.

DISCUSSION

Hoplodactylus duvaucelii had a prehuman distribution very similar to that of tuatara, *S. punctatus* (Hay et al., 2008). Other New Zealand gekkonid lizards (of the genera *Woodworthia, Naultinus, Dactylocnemis, Tukutuku, Toropuku, Mokopirirakau*) coexist with mammalian predators, but the large size of *H. duvaucelii* may render it more vulnerable to rodent predators (Hoare, 2006). Today, tuatara share with *H. duvaucelii* a distribution restricted to offshore islands near North Island. Genetic analysis of mtDNA from extinct and extant tuatara populations reveals subdivision of regions, but mainland and island populations show little divergence (Hay et al., 2008). Extinct populations on North Island were genetically more similar to extant northern island populations than to extant southern (Cook Strait) populations (Hay et al., 2008, 2009). In *H. duvaucelii*, we see a similar north–south structuring of populations. The concordance of morphology, mtDNA, and nuDNA suggests the possibility of a past cline across a previously continuous distribution.

Origin of the Maungatautari Specimen.—Removal of nonnative predators from habitat fragments in New Zealand has led to subsequent detection of previously unrecorded native species (Towns and Broome, 2003). For example, the first *Woodworthia maculata* on Tiritiri Matangi Island was detected 13 years after the eradication of kiore (*R. exulans*), and at Maungatautari, a population of threatened endemic New Zealand frogs (*Leiopelma hochstetteri*) was discovered after the construction of a predator-proof fence and eradication of mammalian pests (Baber et al., 2006). However, because the last sighting of *H. duvaucelii* on mainland New Zealand occurred more than 60 yr ago, it seemed unlikely that *H. duvaucelii* could have survived undetected. However, the specimen from Maungatautari is clearly *H. duvaucelii*. MtDNA provides strong evidence that this specimen is not the offspring of a maternal Cook Strait Islands gecko. Although the mtDNA haplotype is unique, our sample sizes are too small and the level of genetic differentiation is not great enough to completely exclude the possibility that the Maun-

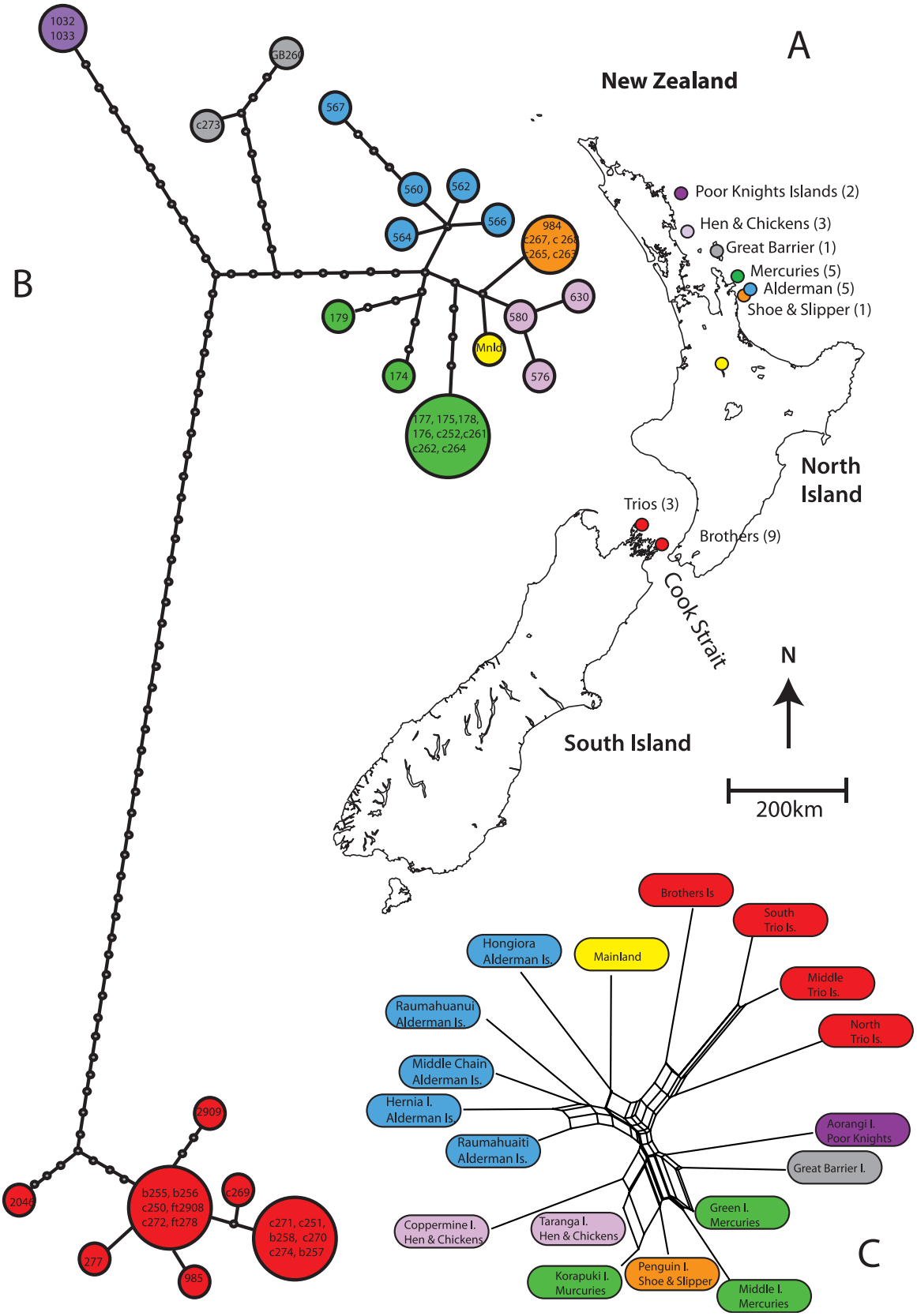

FIG. 3. Genetic structure within the New Zealand *Hoplodactylus duvaucelii* is concordant with their location on offshore islands. Identified island groups (A) and sample size is included in brackets. A minimum spanning network of mtDNA haplotypes, 1,900 bp (B). Circle size corresponds to haplotype frequency, and sample IDs match those presented in Table 1. Inferred but unsampled haplotypes are indicated by small circles, and captive specimens are indicated with prefix "c." The 7-locus NeighborNet splits tree network (C). Genetic distances were inferred from pairwise F_{ST} (captive geckos excluded). The Maungatautari individual is colored yellow.

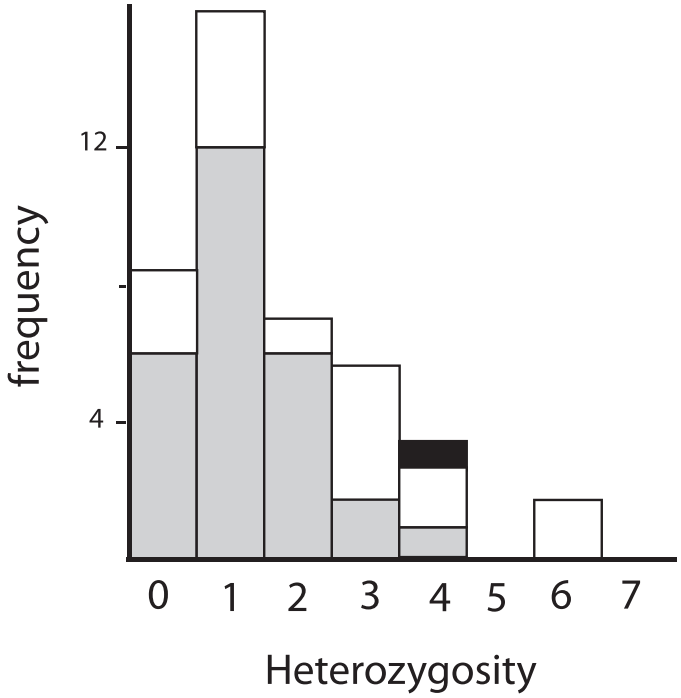

FIG. 4. Heterozygosity levels of wild (grey) and captive (white) *Hoplodactylus duvaucelii* derived from seven nuclear loci. Note relatively high heterozygosity in the mainland New Zealand specimen (black). A captive animal resulting from crossing northern and southern (Cook Strait) parents was heterozygous at six loci.

gatautari gecko originates from within a northern island population. However, all *H. duvaucelii* from the Mercury Island group (including Korapuki) differ by at least six nucleotide substitutions from the Maungatautari haplotype, effectively ruling out the translocated population on Tiritiri Matangi Island as the source of this animal. We would not expect a mainland individual to have an mtDNA haplotype differing substantially from the unique variant described here. D-loop sequence of tuatara mtDNA from extinct populations on the mainland at Waitomo (150 km southwest) was not distinguishable from island haplotypes and differed from them by less than within-island variability (Hay et al., 2008).

The Maungatautari specimen possessed alleles at the RAG-1, Hduv04, and Hduv05 nuclear loci, which were not observed in the northern island populations. The Maungatautari animal possessed private alleles, and alleles otherwise observed in Cook Strait, and northern populations. We anticipate that this combination could result from two possibilities. It could originate from a natural mainland population retaining allelic diversity not retained (or observed) on islands or from a captive cross of northern and southern parentals. The assumption that admixture of mtDNA and nuDNA would result from a cross between northern and southern individuals was supported by some genotypes inferred from captive animals; this pattern is what we would expect if the Maungatautari animal was an intraspecific hybrid of captive origin. We consider this second possibility considerably less likely than a natural mainland population because the mtDNA haplotype and some nuclear alleles of the Maungatautari specimen were not observed in any captive animals genotyped by us.

Because our sampling included only a single individual from most island populations (12/17) we interpret population genetic patterns with caution. Although small samples of 1–3 individuals can be informative given sufficient numbers of loci (Prunier et al., 2013), numbers of inferred clusters may be artificially low if sample sizes are small (Fogelqvist et al., 2010). Had our sample from Maungatautari consisted of 10 individuals, the inferred population clusters may have been greater. In addition, the underlying Bayesian approach used by STRUCTURE is not well suited to data that follows an isolation-by-distance model, as observed here (Pritchard et al., 2007; Schwartz and McKelvey, 2009). When individual genotypes were assigned to two groups (as suggested by the optimal $K = 2$), the Maungatautari specimen showed evidence of northern and southern population admixture, as expected. When individuals were assigned to three population clusters ($K = 3$), then the Maungatautari specimen was assigned (with 0.8 probability) to the Alderman Island genetic cluster. The Alderman Islands are geographically one of the closest extant populations. Thus, we favor the conclusion that the Maungatautari individual is derived from a relictual mainland population, with the caveat that our genetic data are not unequivocal. It is conceivable that a relictual mainland population could retain the genetic diversity detected here. If the unique specimen does represent a surviving population, we note that the current population bottleneck may have lasted only a few generations since the arrival of ship rats and Norway rats. *Hoplodactylus duvaucelii* has longevity of at least 36 yr in natural populations (Thompson et al., 1992), and the species is difficult to detect when present at low densities (Towns and Broome, 2003; Hoare, 2006; CSK, RAH, pers. obs.).

Our data demonstrate levels of genetic and morphological divergence between the northern and Cook Strait clades equivalent to or greater than interspecific distances in some other New Zealand gecko genera such as *Naultinus* (Nielsen et al., 2011). However, the blend of morphological and genetic character states in the Maungatautari specimen indicates that in this case the extant subclades were formerly linked by a mainland metapopulation and should not be considered separate species.

Until the size of the *H. duvaucelii* population at Maungatautari can be established, management plans should not be implemented. We recommend a number of approaches to confirm the presence of *H. duvaucelii* in the Maungatautari reserve. Although changes in gecko behavior following removal of predators can be associated with rapid increases in apparent abundance, genuinely increased population size likely takes more than 10 yr (Hoare, 2006). Targeted nocturnal searches, baited tracking tunnels, and artificial refuges are recommended to allow long-term detection. Closed-cell foam covers fixed to the trunks of mature forest trees create permanent daytime refuges suitable for several species of arboreal New Zealand geckos (Bell, 2009). Future management strategies might include assisted dispersal among populations or maintenance of distinct conservation management units, as practiced for tuatara, depending on geographic scale.

The discovery of a single *H. duvaucelii* individual in a mainland forest of North Island New Zealand suggests the possibility that other relictual populations may exist. Mature forest elsewhere in the country may harbor populations on the verge of extinction; all possible effort for controlling introduced mammalian predators is warranted (Green, 2011). In this study, we have elucidated significant genetic variability present in captive colonies. Some captive colonies possess distinct haplotypes and private alleles, which should be maintained for long-term conservation genetic viability of the species, even if the origins of the individual animals are unclear.

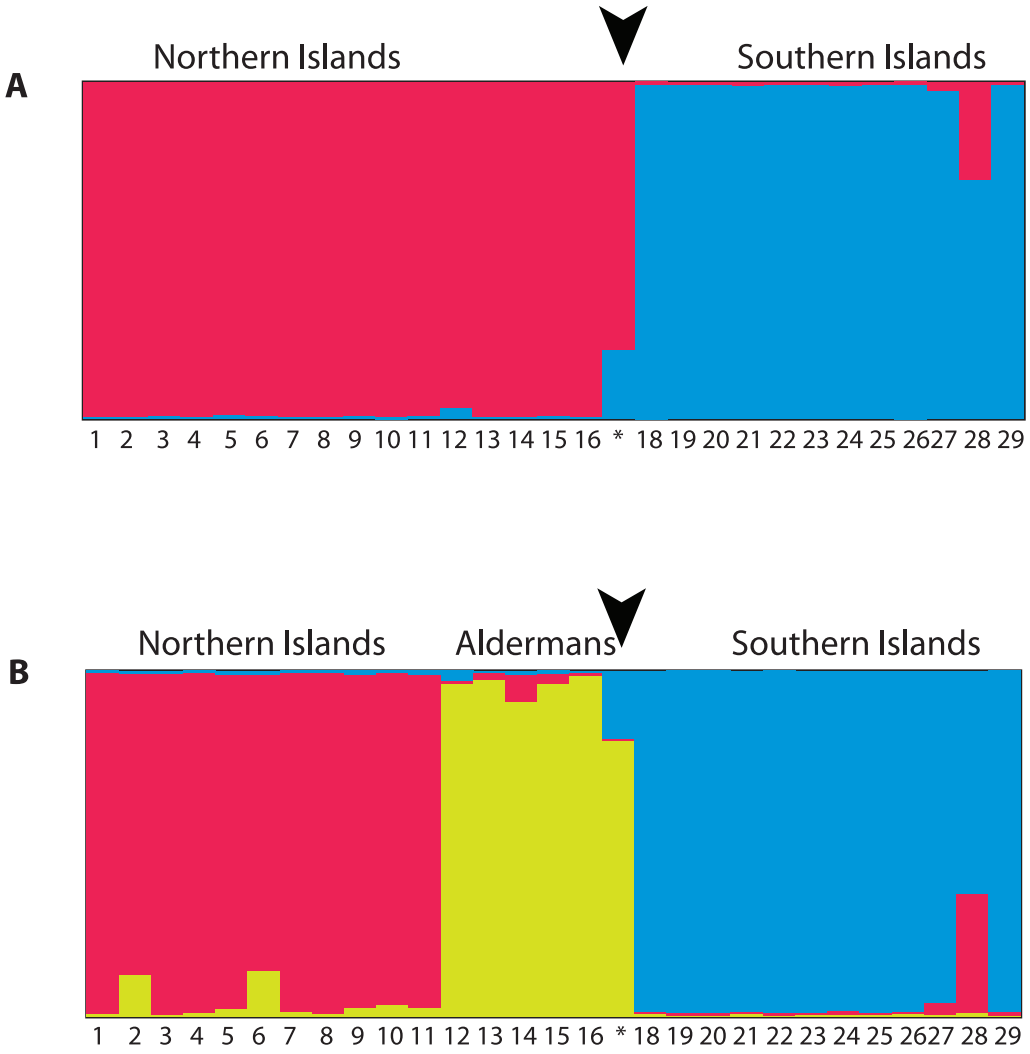

FIG. 5. Genetic structure within the New Zealand gecko *Hoplodactylus duvaucelii* inferred from seven nuclear loci using STRUCTURE analysis. (A) K = 2; (B) K = 3. Maungatautari individual indicated with arrow and *. Localities are: Poor Knights (1), Hen and Chickens (2–4), Great Barrier (5), Shoe and Slipper (6), Mercuries (7–11), Aldermans (12–16), Brothers (18–26), Trios (27–29).

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Supplementary Data

Supplementary data associated with this article can be found online at http://dx.doi.org/10.1670/13-128.s1.