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Phylogenetic placement of a recently discovered population of the threatened alpine she-oak skink *Cyclodomorphus praealtus* (Squamata: Scincidae) in Victoria

JOANNA SUMNER^{1*}, MARGARET L. HAINES¹, PETER LAWRENCE², JENNY LAWRENCE³ AND NICK CLEMANN⁴

¹ Museums Victoria, GPO Box 666, Melbourne VIC 3001

² 2 Rose Street, Heyfield VIC 3858

³ Parks Victoria, Licola Road, Heyfield VIC 3858

⁴ Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, PO Box 137, Heidelberg VIC 3084 (Nick.Clemann@delwp.vic.gov.au)

* to whom correspondence should be addressed (jsumner@museum.vic.gov.au)

Abstract

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The alpine she-oak skink *Cyclodomorphus praealtus* is a threatened alpine endemic lizard from the mainland of Australia. The species is previously known from disjunct populations in Kosciuszko National Park in New South Wales and three isolated localities in the Victorian Alps. The New South Wales and Victorian populations represent separate evolutionarily significant units. In 2011, a fourth Victorian population was discovered. We conducted a phylogenetic analysis and determined that the newly discovered population is discrete and may have been separated from other populations since the end of the last glacial maxima. This population requires separate management.

Keywords

Cyclodomorphus praealtus, phylogenetic relationship, conservation, threatened species

Introduction

The alpine she-oak skink *Cyclodomorphus praealtus* Shea (1995) is restricted to sky island plains (*sensu* Koumoundouros et al., 2009) above 1500 m elevation in the mainland Australian alpine bioregion (Cogger, 2014). The species has been recorded in New South Wales (NSW) at Kosciuszko National Park and in Victoria at the Bogong High Plains, around Mt Hotham, Mt Higginbotham and Mt Loch, and on the Dargo High Plains at Lankey and Omeo Plains (Clemann, 2011; fig. 1). The Victorian sites are all within approximately 30 km of each other (Table 1). The species has specific habitat requirements, occurring in alpine heathland and alpine grassy heathland. These habitats generally have a disjunct occurrence and are within a matrix of other alpine and sub-alpine habitats in which the alpine she-oak skink is not known to occur. Within some locations, the species distribution is further fragmented by ski resort buildings and groomed ski slopes, roads and tracks. The species is listed as nationally endangered under the federal *Environment Protection & Biodiversity Conservation Act 1999*, threatened in Victoria under that state's *Flora and Fauna Guarantee Act 1988*, critically endangered in Victoria by the Department of Sustainability and Environment (2013) and endangered in NSW under the *Threatened Species Conservation Act 1995*.

The species is considered at risk from climate change (Clemann, 2003; Koumoundouros et al., 2009) and is a high priority for conservation management.

Populations of *C. praealtus* in NSW and Victoria are reciprocally monophyletic at the mtDNA ND4 loci, with a 4.28% net sequence divergence between regions, and it was recommended that they be treated as separate evolutionarily significant units (Koumoundouros et al., 2009). The three known Victorian populations exhibit strong genetic structure, with no current inter-population gene flow, despite evidence for historical connectivity (Koumoundouros et al., 2009). This concurs with their present disjunct distribution on high elevation plateaux and peaks in alpine meadows that are separated by valleys of eucalypt forests.

In 2011, *C. praealtus* was discovered at a fourth Victorian locality – the Wellington Plains (Clemann et al., 2016). This is the southern-most area where this species has been recorded and is 58 km from the closest Victorian population at Lankey Plain. Here we present a phylogenetic analysis to determine the relationship between this new population and those studied by Koumoundouros et al. (2009). Our results provide information that will assist the conservation management of this species.

Table 1. Divergence among five populations of *Cyclodomorphus praealtus* in Victoria (VIC) and New South Wales (NSW). Uncorrected p-distance (number of base substitutions per site averaged over all sequence pairs between groups; bottom) and straight-line distance (km) between locations (top).

	Bogong High Plains, VIC	Mt Hotham, VIC	Lankey Plain, VIC	Wellington Plains, VIC	Mt Kosciuszko, NSW
Bogong High Plains, VIC	–	31	29	96	101
Mt Hotham, VIC	0.01	–	34	71	117
Lankey Plain, VIC	0.012	0.009	–	58	126
Wellington Plains, VIC	0.016	0.019	0.018	–	193
Mt Kosciuszko, NSW	0.052	0.052	0.055	0.059	–

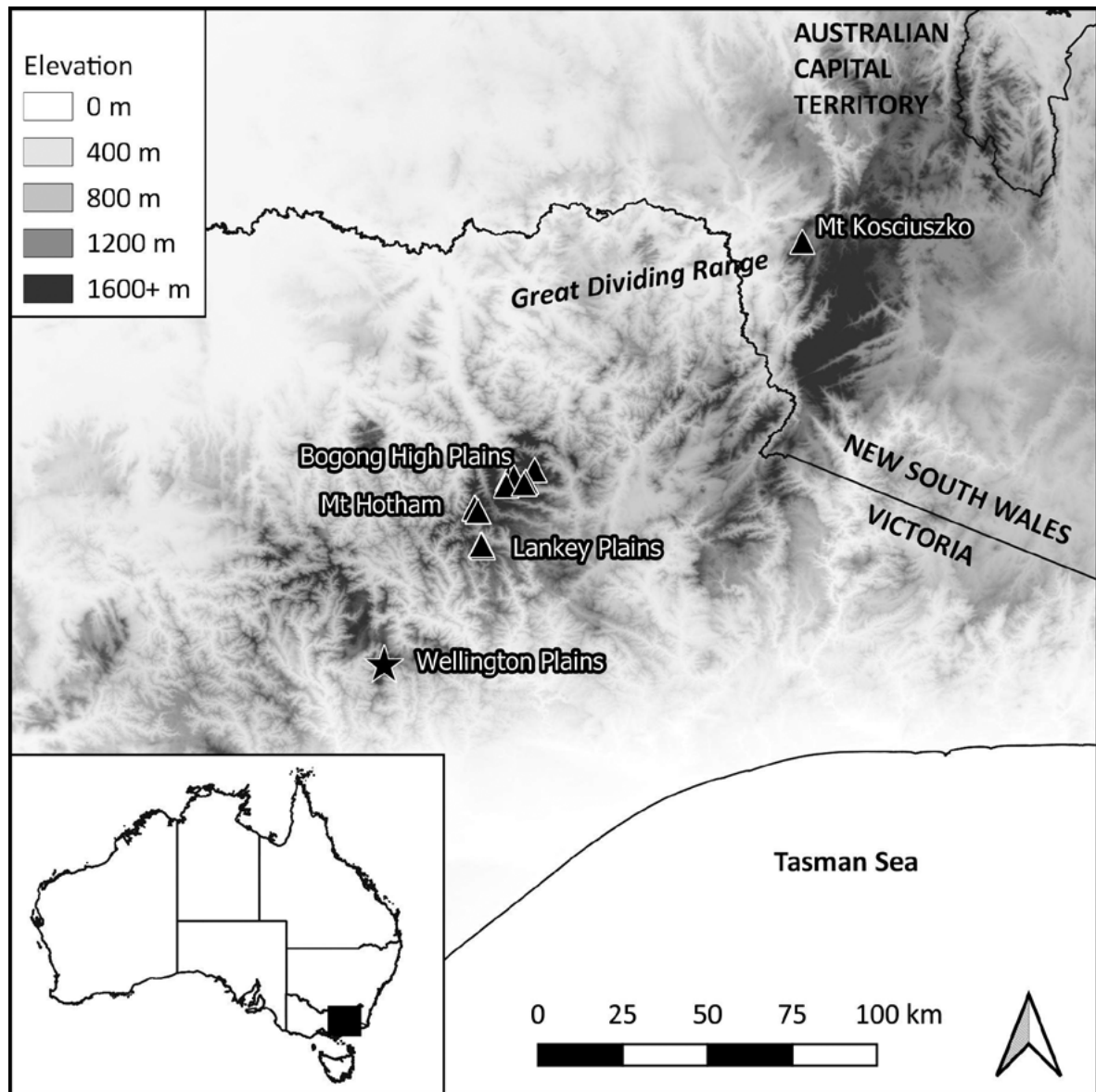


Figure 1: Elevation map of the mainland Australian alpine region indicating the five major populations of *Cyclodomorphus praealtus*, including the new location of Wellington Plains. Elevation is indicated by light, mid and dark grey areas at 500 m intervals, with highest elevations at or above 1500 m above sea level. Adapted from fig. 1; Koumoundouros et al. (2009).

Methods

Discovery of this population of *C. praealtus* and collection of tissue samples is described by Clemann et al. (2016). Tissue samples (tail tips) were collected from two sub-adult *C. praealtus* from the Wellington Plains (1530 m elevation; fig. 1) in November 2011.

We extracted genomic DNA using a Qiagen DNeasy® Blood and Tissue Kit according to the manufacturer's instructions (www.qiagen.com). An 850 base pair region of the mtDNA NADH dehydrogenase subunit 4 (ND4) gene was chosen for sequencing because it has useful levels of variability in this species and in related skink groups (Chapple and Keogh, 2004; Koumoundourous et al., 2009). ND4 was amplified according to the protocols and conditions in Koumoundourous et al. (2009) using the primer pair ND4I

(5' TGACTACCAAAAGCTCATGTAGAAGC 3'), and tRNA-leu (5' TACTTTTACTTGGATTGACCA 3'; Chapple and Keogh, 2004). The resulting polymerase chain reaction product was purified using ExoSAP-IT® (Affymetrix), and the purified products were sent to Macrogen (South Korea) for sequencing.

Forward and reverse sequences were assembled in Geneious R6.1.8 (Biomatters Ltd.) and were aligned against 103 published sequences and outgroups for this species (Koumoundourous et al., 2009) using default settings in ClustalW. We translated the protein-coding regions into amino acids and did not observe stop codons or indels. IUPAC ambiguity codes were used for heterozygous sites. We trimmed the alignments to 758 base pairs because the end of the sequences could not be aligned due to the length-variable loops and stems of the tRNA genes. The Wellington Plains sequences were deposited in GenBank.

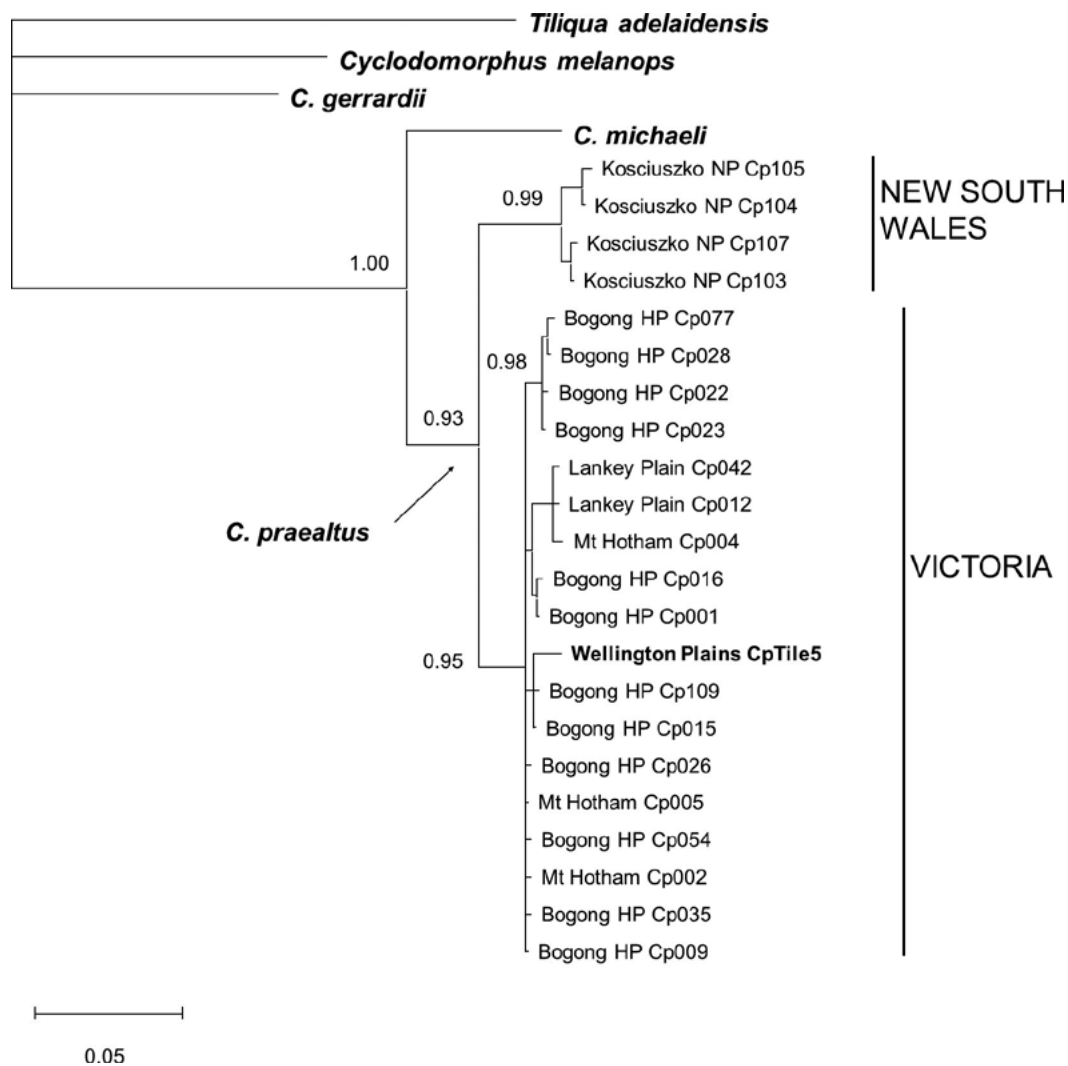


Figure 2: A Bayesian consensus tree of the mtDNA ND4 sequences, depicting the relationship between unique haplotypes at each locality (Bogong High Plains, Lankey Plain, Mt Hotham, Wellington Plains and Kosciuszko National Park) for *Cyclodomorphus praealtus*. Bayesian posterior probabilities are shown at major nodes.

To determine the relationship of the Wellington Plains population to all other known populations, we created a phylogenetic tree. Only unique haplotypes were used. We used Modeltest 3.7 (Felsenstein, 1985) with the PAUP* plug-in run within Geneious R6.1.8 to determine which of the 56 potential models of DNA sequence evolution was most suitable for our data. The GTR+G model of evolution was selected as the most appropriate model using the Akaike information criterion (Akaike, 1974). The MrBayes version 3.2.6 (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003) plug-in within Geneious R6.1.8 was used to conduct a Bayesian Markov chain Monte Carlo phylogenetic analysis using two million generations sampled every 500 generations, with four heated chains sampling independently and a 25% burn-in. Chain convergence was confirmed by examining the log trace in Geneious and estimated sample size values > 200. The mean between group distance was calculated among all five populations in MEGA6 (Tamura et al., 2006).

To infer the relationship between the mitochondrial ND4 sequences from Wellington Plains and the published haplotypes in Victoria, we created a statistical parsimony network using the program TCS (Clement et al., 2000) using a 95% statistical confidence limit for the maximum number of nucleotide substitutions between two haplotypes.

Results

The ND4 sequences comprised 758 base pairs, including 63 variable sites and 50 parsimony informative sites. The

sequences from the two Wellington Plains individuals were identical and differed from all previously known haplotypes. The Bayesian consensus tree (fig. 2) revealed that the ND4 haplotype from Wellington Plains falls within the monophyletic Victorian lineage and did not group with any other populations. The Wellington Plains haplotype differed from the populations in Victoria by 1.6–1.9% and from the NSW population by 5.9% (Table 1).

A haplotype parsimony network of all ND4 sequences generated in TCS (fig. 3) indicates that the Wellington Plains haplotype forms a third distinct haplogroup cluster, separate from all other Victorian haplotypes, and that they cluster more closely with the north-eastern haplogroup that includes individuals from Bogong High Plains and Mt Hotham. Eight nucleotide changes separate the Wellington Plains sample from the closest haplotype from the Bogong High Plains.

Discussion

Analysis of mtDNA ND4 sequences of two *C. praealtus* individuals from Wellington Plains indicates the presence of a haplotype that is not found in any other population across the restricted range of this alpine endemic species. Further work using multiple nuclear genes is needed before we can confirm that the Wellington Plains population is isolated from the other Victorian populations as the current data suggests. It is not possible to determine the level of genetic diversity within the Wellington Plains population due to the small sample size.

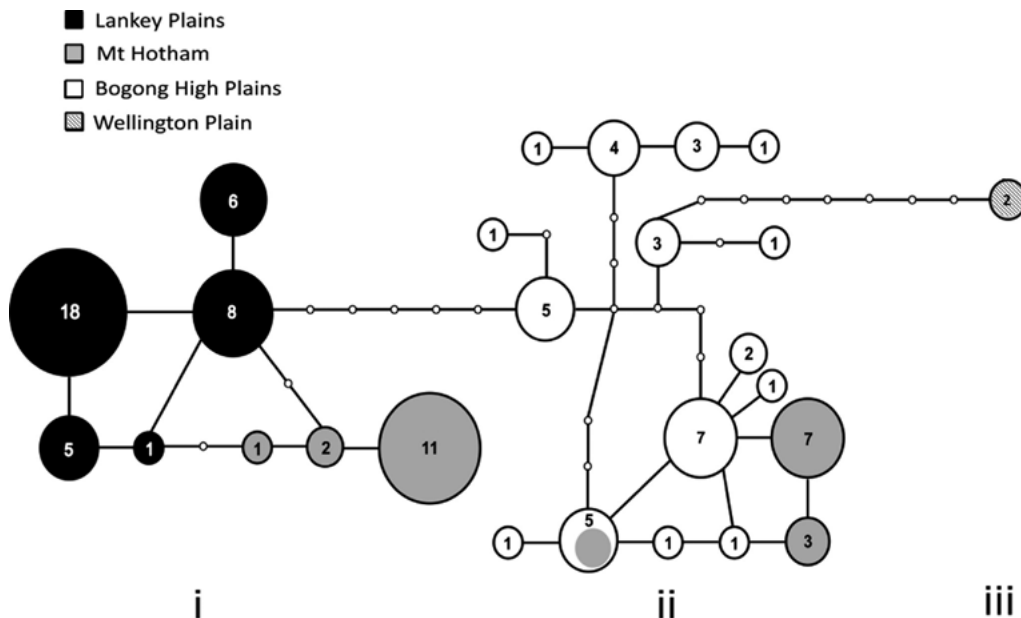


Figure 3: Population structure of Victorian *Cyclodomorphus praealtus* according to ND4 mitochondrial haplotype network. Black indicates individuals from Lankey Plain, dark grey indicates those from Mt Hotham, white indicates those from Bogong High Plains and diagonal stripes indicate those from Wellington Plains. The network structure indicates 3 haplogroups (i, ii and iii) within Victoria. Each circle represents a unique haplotype, with the circle size indicative of frequency and sample sizes within each circle. Empty circles represent missing haplotypes and differ by one base pair from the closest haplotype.

The Wellington Plains population is the most divergent of the populations in Victoria, with 1.6–1.9% sequence divergence from the other populations, compared with the 0.9–1.2% divergence among the other Victorian populations. Using the conventional vertebrate mtDNA clock calibration of 2% sequence divergence per million years (Avice, 2004), suggests a divergence time between Wellington Plains and the other populations in Victoria of between 500 000 and one million years before present. This places the divergence during the late Pliocene to early Pleistocene, during which climatic oscillations intensified resulting in rapid fluctuation between cool-dry and warm-wet conditions across south-eastern Australia (Markgraf et al., 1995). However, this predates the most recent glacial period from approximately 25 000–18 000 years before present, during which the alpine habitat extended into the valleys connecting the mountain peaks, allowing gene flow between populations of other alpine endemics.

In 2019 severe bushfires burned across the entire Wellington Plains plateau and, as a result, the status of this population is now uncertain. Surveys to determine whether the population still remains on the Wellington Plains are an urgent priority, both to confirm the status of this population and to identify any other specific threats. Threats already identified for *C. praealtus* in the Victorian Alps include climate change, loss and degradation of habitat (due to a range of processes, including several severe fires in the last decade, construction and maintenance of infrastructure and roads, grazing and trampling by exotic herbivores, and recreational activities), and predation by exotic carnivores (Clemann, 2003, 2013). Historically, loss and degradation of the habitat of *C. praealtus* due to cattle grazing also occurred in the Alpine National Park, and damage from this impact remains evident in parts of this lizard's range in Victoria (N. Clemann pers. obs.). Other reptiles endemic to the mainland alpine region also occur on the Wellington Plains, including threatened species such as the alpine bog skink *Pseudemoia cryodroma* and the tussock skink *P. pagenstecheri*. The fact that this population of *C. praealtus* remained unknown until so recently demonstrates that further surveys of Victorian alpine environments are needed if we are to document and effectively manage the biodiversity of this region, especially in the face of increasingly frequent and devastating bushfires.

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