Variation in thermal biology of three closely related lizard species along an elevation gradient

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The critical thermal limits of organisms and the thermal sensitivity of their performance tend to vary predictably across latitudinal gradients. There has been comparatively less investigation into variation in thermal biology with elevation, despite similar gradients in environmental temperatures. To redress this, we examined critical thermal limits (CTmin and CTmax), thermal sensitivity of locomotor performance, and shelter site attributes, in three lizard species that replace one another along a contiguous elevation gradient in south-eastern Australia. The species examined consisted of a highland specialist, *Liopholis guthega*, mid-elevation species, *Liopholis montana*, and lowland species, *Liopholis whitii*. We found similar habitat attributes between the species, but *L. guthega* predominantly occurred in open habitat, which might reflect a strategy for maximizing exposure to insolation. We found intraspecific variation in lizard thermal traits, most notably in cold tolerance of *L. guthega* and in both heat and cold tolerance of *L. whitii*, suggesting population-specific variables acting on thermal physiology rather than a species distribution maintained by distinct thermal tolerances. This study represents one of the few examinations of thermal trait variability within and between species with elevation in a temperate system and provides evidence for thermal physiology driven by adaptation and/or physiological plasticity to local conditions.

ADDITIONAL KEYWORDS: alpine – distribution range – *Liopholis* – montane – reptile – skink – thermal tolerance.

INTRODUCTION

Understanding the link between geographical range, physiology and temperature is fundamental in the field of macro-ecology, yet the mechanisms responsible for patterns in species distribution remain poorly understood (Caldwell *et al.*, 2015; Oyen *et al.*, 2016). This link is increasingly relevant because widespread range shifts are predicted as an outcome

of climate change, with ectotherms likely to be most affected (Kearney *et al.*, 2009; Sinervo *et al.*, 2010; Böhm *et al.*, 2016; Winter *et al.*, 2016). Dependent on external heat sources, ectotherms must maintain body temperatures within a narrow range essential for performance by adjusting thermoregulatory behaviours (Caldwell *et al.*, 2017) or through long-term changes in thermal sensitivity through acclimatization or adaptation, or both (Gvoždík & Castilla, 2001; Sheldon & Tewksbury, 2014; Llewelyn *et al.*, 2018). The experimental establishment of the upper and lower thermal limits [also referred to as

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critical thermal maximum (CTmax) and critical thermal minimum (CTmin)] of an organism allows us to estimate this range (Sheldon & Tewksbury, 2014). The CTmin and CTmax are the temperatures at which an organism loses critical motor function and the ability to escape from conditions that could lead to death (Oyen *et al.*, 2016).

In ectotherms, behavioural thermoregulation can shield some physiological traits from selection (the Bogert effect) more so than others, which cannot be as easily buffered and are thus exposed to stronger selection (Muñoz *et al.*, 2014). In diurnal species, cold temperatures cannot be buffered at night as easily as hot temperature can be during the day (Muñoz *et al.*, 2014). Trends in critical thermal maxima are therefore not as pronounced as in critical thermal minima, and upper thermal limits (compared with lower thermal limits) are typically more conserved among populations (Moritz *et al.*, 2012; Kellermann *et al.*, 2012; Pontes-da-Silva *et al.*, 2018) and species (Addo-Bediako *et al.*, 2000; Araújo *et al.*, 2013).

Long-term fluctuations in temperature, such as those experienced across environmental gradients, are likely to lead to evolutionary processes acting to widen the breadth of thermal tolerance (Gvoždík & Castilla, 2001; Sheldon & Tewksbury, 2014), leading to observable geographical patterns in thermal tolerances, such as decreases in critical thermal minima with increasing latitude (Gaston & Chown, 1999; Addo-Bediako *et al.*, 2000; Oyen *et al.*, 2016). Organisms that occur across elevation gradients also experience extreme fluctuations in seasonal and daily temperatures over comparatively short geographical distances, and differences in environmental temperature are highly pronounced across small spatial scales, resulting in strong elevational stratification of habitat, thermal niches and species endemism at different elevations (Dirnböck *et al.*, 2011; McCain & Colwell, 2011; Gifford & Kozak, 2012; Clusella-Trullas & Chown, 2014; Oyen *et al.*, 2016). Despite this, very little has been done to examine thermal tolerance variability and elevation (Gaston & Chown, 1999; Oyen *et al.*, 2016), and the factors underlying species elevational range limits remain unknown for most organisms (Gifford & Kozak, 2012; Rodríguez-Castañeda *et al.*, 2017). Detailed studies on closely related species across elevation gradients allow us to examine the link between thermal tolerance, elevation and distribution, and present an opportunity to study environmental factors limiting populations locally.

Furthermore, studies that model the response of a species to climate change using thermal tolerance measures are becoming more prevalent in the literature (Caldwell *et al.*, 2015; Wright *et al.*, 2016; Atkins, 2018; Herrando-Pérez *et al.*, 2018). These models are frequently built on estimated critical thermal limits of one population per species (Herrando-Pérez *et al.*, 2018). Underlying this methodology is the assumption that intraspecific variation in thermal tolerances is negligible compared to interspecific variation and is unlikely to effect overall patterns (Herrando-Pérez *et al.*, 2018). In order to evaluate interspecies vulnerability to climate change comprehensively, there is a need to incorporate thermal biology measures across the geographical range of species. Studies that examine clinal variation in intraspecific thermal tolerance are an important component in better understanding interspecific variation. However, studies have tended to examine variation in one species over an elevation gradient (Gvoždík & Castilla, 2001) or in multiple species collected from different elevations, and understanding of intraspecific differences relative to interspecific differences is lacking (Huang *et al.*, 2006; Caldwell *et al.*, 2015). Temperate systems are often harsher and more seasonal than their tropical counterparts (Barve & Dhondt, 2017; Bastianelli *et al.*, 2017). Thus, ectotherms living at high elevations in high latitudes are expected to have relatively wide tolerance breadths driven by cold tolerance compared with lowland species and tropical species (Clusella-Trullas & Chown, 2014; Oyen *et al.*, 2016), yet temperate systems are also poorly represented in the literature.

The Australian scincid lizard genus *Liopholis* includes three species occurring along an elevation gradient in temperate south-eastern Australia. *Liopholis guthega* is the continent's highest elevation reptile (Atkins, 2018), occurring in two restricted regions from 1600 m above sea level (a.s.l.) up to 2170 m. The narrow elevational band occupied by this species abuts the ranges of its closest relatives, *Liopholis montana* and *Liopholis whitii*, two species that extend to lower elevations (Donnellan *et al.*, 2002; Chapple & Keogh, 2004; Chapple *et al.*, 2005; Fig. 1). The mountain skink (*L. montana*) occupies subalpine to montane environments (between 1800 and 900 m) throughout the southern Great Dividing Range (Donnellan *et al.*, 2002). White's skink (*L. whitii*) is a widespread species occurring throughout eastern Australia, from sea level to 1400 m (Wilson & Swan, 2010; W. Osborne, unpublished data). Thus, this species group provides an excellent model to investigate ecological and physiological trait variability with elevation. Both the high and mid-elevation species experience extended snow cover for several months of the year, with the activity season decreasing in length at higher elevations. The genetic split between the three is thought to have occurred in response to rapid cooling and drying of southeast Australia during the late Miocene to Pliocene (2–5 Mya; Chapple & Keogh, 2004; Chapple *et al.*, 2005). It is likely that the *Liopholis* species
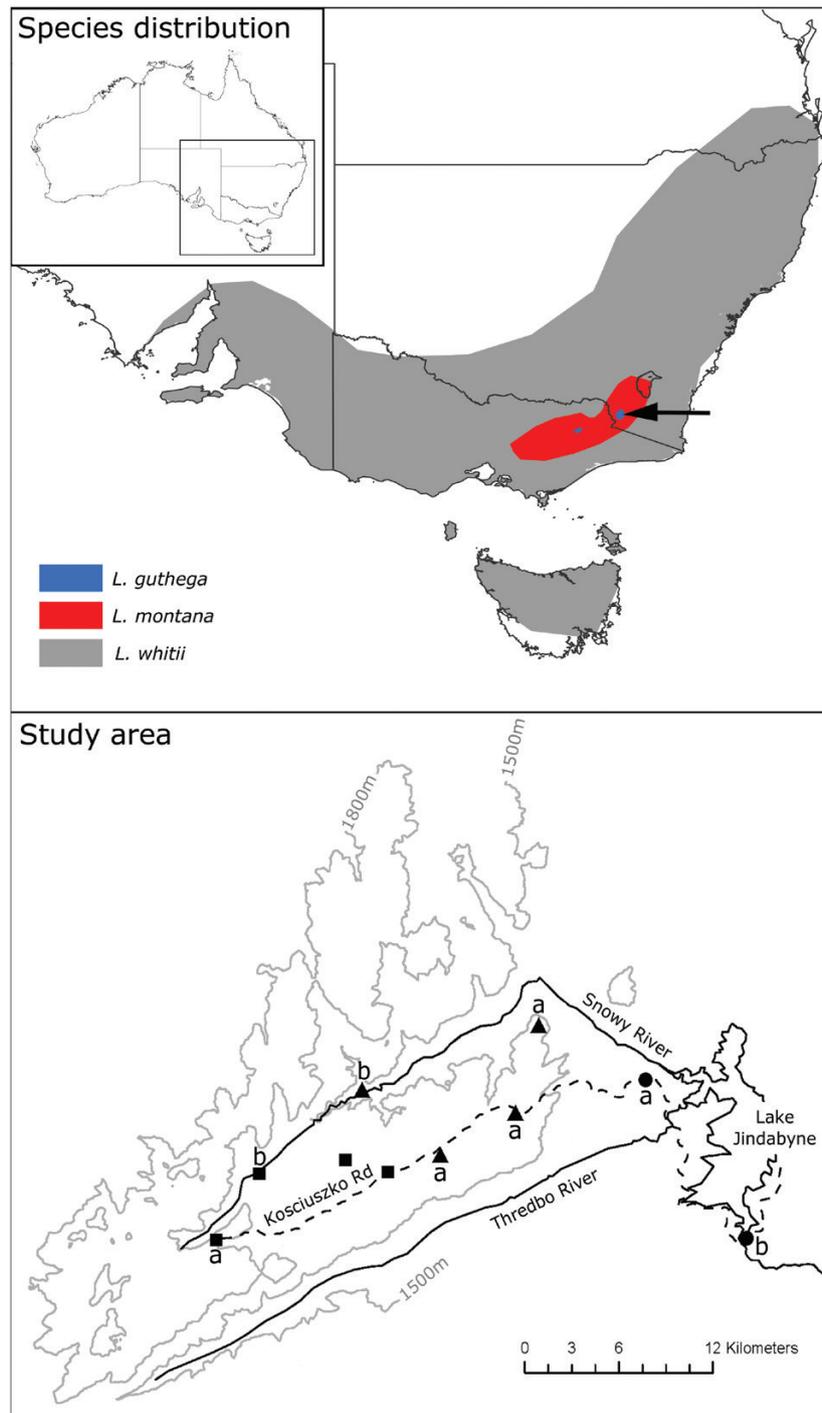
Figure 1. Map showing contrasting broad-scale distribution of *Liopholis* species in south-eastern Australia, with detail of study area showing localities where *Liopholis* populations were found along an elevational cline in Kosciuszko National Park. Symbols are as follows: squares, *Liopholis guthega*; triangles, *Liopholis montana*; circles, *Liopholis whitii*. Populations of each species used in thermal assays are denoted by: a, highest population; b, lowest population. An additional two *L. guthega* populations not sampled have been included to illustrate the spatial proximity of *L. guthega* and *L. montana* populations within the study area.

distributions have radiated and contracted several times with the alpine zone during subsequent Pleistocene glacial cycles (Chapple *et al.*, 2005). The three species are similar morphologically and most probably occupy similar ecological niches, appearing to be largely allopatric in occurrence despite some broader scale overlap in elevational range.

There are concerns that changing environmental conditions associated with climate change might erode species' thermal niches and increase likelihood of secondary contact. The range-restricted alpine endemic, *L. guthega*, is likely to experience an expansion of activity season (Atkins, 2018), but a limited dispersal capacity might leave it vulnerable to habitat incursion by other *Liopholis* (Atkins, 2018). Here, we investigate distribution and habitat parameters and examine intra- and interspecific variation in thermal physiology to understand the drivers of elevational distribution in these species. If discrete thermal tolerances are driving the species' distribution, we would expect a species difference in CTmin, with mid-elevation and lowland species constrained from high elevations by a lesser tolerance to cold compared with the high-elevation congener. A difference in critical thermal minima may correspond to thermal dependence of performance, with high-elevation species able to perform better at lower temperatures. There might also be a species difference in CTmax, with the higher-elevation species, *L. guthega* and *L. montana*, being less tolerant to warm temperatures experienced over longer periods at lower elevations, thus limiting their distribution.

MATERIAL AND METHODS

HABITAT AND DISTRIBUTION

Surveys for *Liopholis* populations were undertaken between 920 and 1860 m a.s.l., between November and March in 2016–2018, to identify elevational distributions and any occurrences of sympatry. Surveys targeted previous species records (Atlas of Living Australia, 2016), which were located primarily in areas with granite outcrops and subsurface boulders in open snow gum (*Eucalyptus pauciflora*) woodland or tall open forest (Donnellan *et al.*, 2002; Chapple *et al.*, 2005; Atkins, 2018). Searches were extended from these points, following topographic contours up to 5 km from records. Based on these surveys, we identified a high- and low-elevation populations for each species, which were then used for the sampling component of the study. To limit confounding seasonal effects on thermal physiology, sites that were closest in elevation were sampled within 1 month of each other (Table 1). Seasonal variability in critical thermal tolerances has been examined in the low-elevation population of *L. whitii* (our unpublished data). We found that CTmin increased monthly throughout the active season but CTmax did not. The range of CTmin recorded for *L. whitii* (low elevation) throughout the season was much higher than CTmin recorded for *L. guthega* at the highest sample site along the gradient, and lower than CTmin recorded for *L. whitii* (high-elevation site), reflecting the main findings of this study. Thus, we believe that the results presented here are indicative of true patterns rather than seasonal variability of critical thermal limits. We collected both males and females for two reasons. First, rarity of *L. guthega* (Endangered; Clemann *et al.*, 2018) and *L. montana* (Near Threatened; Clemann *et al.*, 2018) meant that both sexes were needed to maximize sample size. Second, we saw an opportunity to undertake a 'between-sex' comparison, albeit only between gravid females and males, because all captured females were gravid. Skinks were caught by hand or with the aid of a noose pole, from the entrance to burrows or sheltering crevices.

Attributes of the habitat surrounding these active shelter sites were recorded by placing a 5 m² quadrat, divided into four 1.25 m² subregions, at the location of each collected skink. The percentage cover of rock,

Table 1. Collection site details presented from lowest to highest elevation

Species	Elevation (m a.s.l.)	Capture month	Snout–vent length (cm; mean ± SE)	*N* (female:male)
Liopholis whitii	920–930	November	77.25 ± 0.99	20 (12:8)
Liopholis whitii	1180–1190	November	75.21 ± 1.54	14 (6:8)
Liopholis montana	1340–1370	March	89.50 ± 2.74	8 (4:4)
Liopholis guthega	1620–1650	February	93.31 ± 1.47	10 (0:10)
Liopholis montana	1640–1660	December	92.33 ± 1.90	10 (4:6), 2 (1:1), 2 (0:2)*
Liopholis guthega	1840–1860	December	98.26 ± 1.21	19 (10:9)

Only *Liopholis guthega* males were collected from the lower-elevation site, because survey times for this site coincided with birthing times for this species. *High-elevation *Liopholis montana* was made up of a composite of three nearby sites from similar elevations.

soil and leaf litter was recorded, as were ground cover (< 30 cm), mid-storey (> 30 cm high) and trees (tree trunks and low-lying branches) for each subregion. The percentage of canopy cover was estimated from a photograph taken directly above the centre of the quadrat. Leaf litter depth (in millimetres) was measured at four points, and total log length within the quadrat was measured (in metres). Substrate temperatures were recorded using temperature-sensitive data loggers (Maxim Thermochron iButtons, DS1922L) at each sample site. Data loggers ($N = 4$) were enclosed in a deflated balloon to provide protection from moisture and placed in areas where lizards had been seen basking. Substrate temperatures were recorded four times a day from June to November 2017.

ANIMAL HUSBANDRY

On the day of capture, skinks were held in cloth bags in the field before transfer to a nearby research facility (36°20′47.8″S, 148°36′20.9″E). On arrival, sex was determined via eversion of the hemipenes of males, and females were palpated to confirm reproductive status (Melville & Swain, 1999). The snout–vent length (SVL) of both sexes was measured to the nearest 1 mm. Skinks were housed individually in plastic enclosures (60 cm × 40 cm × 35 cm) lined with paper towel, with a flat granite rock provided for basking and a plastic hide for shelter. Ambient daytime temperatures ranged from 15 to 25 °C. Skinks were provided with a heat source in the form of a 40 W incandescent light bulb suspended from the roof of the enclosure at one end, providing a thermal gradient of 22–40 °C for a 12 h period (07:00–19:00 h) that allowed for behavioural thermoregulation. Lizards were fed every second day on a diet of mealworms dusted with calcium and vitamin supplements (Vetafarm, Multical dust). Water was available ad libitum.

THERMAL INDICES AND MAXIMAL SPRINT SPEED

The upper and lower thermal limits of lizards were established by estimating the CTmin and CTmax, following protocols of Lutterschmidt & Hutchison (1997a, b). Lizards were given 24 h to acclimate to captivity before beginning CTmin thermal trials, followed by a rest day and CTmax trials. Males and females were used in CTmin trials, but only males were tested in CTmax trials to avoid any risks that heating females might have on unborn embryos. The righting reflex was used to determine CTmin and CTmax (Lutterschmidt & Hutchison, 1997a, b). A single observer (A.F.S.) made all of the measurements, and no adverse impacts on any skink were observed.

To obtain the CTmin and CTmax, each skink was first placed in an incubator set to 15 °C for 1 h.

A thermocouple probe (Eutech Instruments; EcoScan JKT; accuracy ± 0.5 °C) was inserted ~1 cm into the cloaca to determine the starting temperature (15 ± 2 °C) and was then taped (Leukopor tape) around the tail to ensure the thermocouple remained in the cloaca for the entirety of the trial. Each lizard was placed in a clear, lidless, plastic container (25 cm × 13 cm × 23 cm), which was held in either an ice bath for CTmin trials or a water bath heated to 50 °C for CTmax trials. Ramping rate was kept between 1 and 1.5 °C per 2 min. Body temperature was recorded using the thermocouple at 30 s intervals for the duration of the trials. For CTmin, skinks were turned onto their dorsal surface once body temperature dropped to 8 °C. For CTmax, turning began once body temperature reached 37 °C. The tolerance breadth (TTB) was calculated by subtracting CTmin from CTmax (Huey & Stevenson. 1979; Sheldon & Tewksbury, 2014).

We also measured thermal sensitivity of locomotor performance (sprint speed) in both sexes, following a rest day after thermal indice trials. Maximal sprint speed was characterized at three body temperatures experienced by skinks across their active season (15, 25 and 30 °C), to investigate the influence of body temperature on locomotor ability (Sun *et al.*, 2014; Artacho *et al.*, 2015) and to compare locomotor ability within and between species. Skinks were acclimated to temperature for 1 h in an incubator before each temperature trial. All skinks were first tested at 15, then at 25 and lastly at 30 °C, with one temperature trial completed in a day. A thermocouple probe was inserted –1 cm into the cloaca to determine the starting temperature and then removed before commencement of the trial. Skinks were then released at one end of a 1 m racetrack enclosed on the sides, with the bottom lined with sandpaper (fine) to assist lizards with gripping of the substrate. As per Crowley (1985), skinks were chased down the track by the observer's (A.F.S.) hand. Each skink was run three times at each temperature, with the time to complete recorded using a stopwatch. As per Artacho *et al.* (2015), the fastest speed for each temperature was used as the estimate of maximal sprint speed. Maximal sprint speed at each temperature treatment was used to calculate speed (in metres per second; speed = distance/time).

STATISTICAL ANALYSIS

Data analysis was conducted in the statistical program R v.3.4.1 (R Core Team, 2018). For habitat analysis, correlating variables were removed [leaf litter depth, tree cover and log length (canopy cover was retained)], and data were transformed (square root) and run through a principal components analysis with varimax rotation to reduce the data into related principal component (PC) scores. Components were retained

based on the Kaiser–Guttman criterion (eigenvalues ~1; Jackson, 1993). Variables with a loading of ≥ 0.4 were considered to contribute to a component. To test species differences, one-way ANOVAs were conducted on axis scores from the first two axes.

Thermal tolerance measures (CTmin, CTmax and TTB) adhered to assumptions of normality and homogeneity of variance. We used linear models (ANCOVA) to determine thermal tolerance variation between populations (within species at high and low elevations, and between species) and sexes for CTmin, with SVL as a covariate. Tukey's HSD tests determined post hoc differences. For analyses of sprint speed, data were $\log_{10}$-transformed following violation of assumptions. Sprint speeds were compared between sexes and all *Liopholis* populations using a two-way repeated-measures ANCOVA. This was conducted in the package 'lme4', with population and sex as non-repeated-measures factors, temperature treatment as a repeated-measures factor, SVL as a covariate, and skink individual as a random effect to account for use of same skink in multiple treatments. Least squared means comparisons ('lsmeans') were used to determine post hoc differences. It was not necessary to control for animal size because SVL did not affect the critical thermal tolerance limits or the effect of sprint speed in above analyses ($P > 0.05$ in all cases; see Results).

RESULTS

HABITAT AND DISTRIBUTION

Liopholis montana and *L. guthega* were found in closest proximity, with populations of the two located within 2 km of each other (Fig. 1). A population of *L. montana* was located at a site higher in elevation (1660 m) than the lowest recorded *L. guthega* site at ~1620 m. We did not find *L. montana* and *L. whitii* in close proximity along the sampled gradient. The highest population of *L. whitii* occurred at 1180 m, and the lowest *L. montana* at 1370 m.

The principal components analysis containing habitat variables revealed two factors that explained a moderate amount of variation (70.1% of the variance; Table 2). Principal component 1 explained 46.2% of the variance and loaded towards increased canopy cover, reduced ground cover and crevice width. Thus, PC1 mostly represents traits associated with the presence of canopy, in addition to some traits of rock and other vegetation structure. Principal component 2 explained 23.9% of the variance and loaded strongly towards increasing rock cover and reduced mid-storey and soil cover. When examining habitat differences between the species, we found significant differences between species for habitat traits loading onto PC1 ($F_{2,47} = 51.8$,

Table 2. Component loadings of habitat traits observed on two orthogonally rotated principal components (PC1 and PC2)

Habitat trait	PC1	PC2
Canopy cover (%)	0.42*	−0.25
Rock cover (%)	0.37	0.60*
Crevice width (mm)	−0.50*	0.24
Soil cover (%)	0.37	−0.55*
Mid-storey cover (%)	−0.33	−0.45*
Ground cover (%)	−0.44*	−0.13
Percentage of variance explained	46.2	23.9
Total variance explained	70.1	–

*Traits with a factor loading of ≥ 0.4 were considered to contribute to a component.

$P < 0.001$) and PC2 ($F_{2,47} = 5.77$, $P < 0.01$). *Liopholis montana* and *L. whitii* differed from *L. guthega* ($P < 0.001$) for habitat traits on PC1 (Fig. 2). *Liopholis whitii* and *L. montana* differed for habitat traits on PC2 ($P < 0.01$).

THERMAL TOLERANCE

The CTmin differed between populations ($F_{5,70} = 27.743$, $P < 0.001$; Fig. 3), but not between males and gravid females ($F_{2,70} = 1.651$, $P = 0.199$) or their interaction ($F_{4,70} = 1.071$, $P = 0.377$), or with SVL ($F_{1,70} = 1.42$, $P = 0.237$). *Liopholis guthega* collected from 1860 m had the lowest mean CTmin (1.17 ± 0.134 °C), which was significantly lower than CTmin (2.69 ± 0.235 °C) of *L. guthega* collected at 1620 m ($P = 0.021$) and all other *Liopholis* populations (Supporting Information, Table S1). *Liopholis whitti* collected from 1180 m had the highest mean CTmin (5.74 ± 0.416 °C), significantly higher than *L. whitii* collected from 920 m ($P < 0.001$) and all other *Liopholis* populations (Supporting Information, Table S1). We found significant differences in CTmax between populations ($F_{5,32} = 4.011$, $P < 0.001$; Fig. 3), but not with SVL ($F_{1,32} = 0.431$, $P = 0.516$). Population differences were driven by the *L. whitii* (1180 m), which had a much lower CTmax than *L. guthega* ($P = 0.017$) and *L. montana* ($P = 0.006$) at their highest populations (Supporting Information, Table S1). In accordance with these results, tolerance breadth (TTB) differed between populations ($F_{5,32} = 18.51$, $P < 0.001$). Intraspecific differences were found in *L. guthega*, which had a wider tolerance breadth at 1860 m than at 1620 m ($P = 0.017$; Supporting Information, Table S1). *Liopholis whitii* at 1180 m had the narrowest tolerance breadth, significantly narrower than the other *L. whitii* population at 920 m ($P < 0.001$) and all other populations (Supporting Information, Table S1).

MAXIMAL SPRINT SPEED

There were significant differences between lizards run at different temperatures and between lizards from different populations, but not between sexes or with SVL (Table 3). *Liopholis montana* from 1660 m performed the most poorly (Fig. 4; Supporting Information, Table S2), running more slowly than all the populations at one or more of the temperature treatments. At 25 and 30 °C, *L. whitii* (1180 m) ran more slowly than *L. guthega* (1860 m). At 30 °C, this population of *L. whitii* displayed a reduction in performance compared with several other *Liopholis* populations (Fig. 4; Supporting Information, Table S2).

Thermal sensitivity of sprint speed differed with population in some species. In *L. guthega* (Fig. 4A), sprint speed increased significantly between 15 and 25 °C (t_{138} = −6.639, P < 0.001) but not between 25 and 30 °C (t_{138} = −2.356, P = 0.052) in the high population. The low population followed the same pattern, with sprint speed increasing significantly between 15 and 25 °C (t_{140} = −2.849, P = 0.01) but not between 25 and 30 °C (t_{140} = −2.116, P = 0.090). For *L. montana* (Fig. 4B) collected from 1660 m, sprint speed increased between 15 and 25 °C (t_{138} = −4.588, P = < 0.001) but not between 25 and 30 °C (t_{138} = −1.269, P = 0.415). Sprint speed increased significantly between 15 and 25 °C (t_{138} = −3.919, P < 0.05) and 25 and 30 °C (t_{138} = −2.861, P = 0.013) in the lower *L. montana* population. At the highest *L. whitii* population, sprint speed was insensitive to heat treatment and did not change significantly between 15 and 25 °C (t_{138} = −2.257, P = 0.066) and 25 and 30 °C (t_{140} = 2.059, P = 0.102; Fig. 4C). At the lower population, sprint speed increased between 15 and 25 °C (t_{138} = −6.943, P < 0.001) but not between 25 and 30 °C (t_{138} = −1.550, P = 0.271).

DISCUSSION

We have demonstrated that some aspects of *Liopholis* thermal physiology differ over a landscape scale and that this appears to be independent of species boundaries. The two higher elevation species are the most genetically similar, having separated from *L. whitii* in the late Miocene to Pliocene (2–5 Mya) during cooling and drying of the Australian continent (Donnellan *et al.*, 2002; Chapple *et al.*, 2005). Despite this evolutionary history, these two species did not show conserved thermal physiology compared with *L. whitii*. There was overlap between thermal biology of all three species at low to mid-elevations, suggesting that temperature alone is not the environmental factor limiting species distributions. The three species exhibit a distribution where one species replaces another across an elevational cline. A zone of replacement between *L. guthega* and *L. montana*

was identified between 1600 and 1700 m. There are no large-scale physical barriers to dispersal (such as deep valleys) within this zone. Other environmental factors that change over small scales, such as vegetation type, geological and geomorphological features (distribution of rocky outcrops and alpine bogs) and fire history, may play a role in isolation of populations (Atkins, 2018). A specific area where *L. montana* and *L. whitii* might interact was not detected in this survey, but we expect a zone of overlap to occur between 1200 and 1400 m based on the populations located during sampling. Further surveys might identify these two species in closer proximity in the Kosciuszko region. Previous work in other localities has reported the presence of *L. whitii* and *L. montana* populations within 200 m of each other (Scabby Range, ACT; W. Osborne, unpublished data), and *L. montana* occurring as low as 900 m (Victoria; Donnellan *et al.*, 2002), suggesting the potential for interaction between these two species in other localities.

Vegetation structure around burrows and crevice shelter sites differed between the species, in that *L. guthega* occurred in quadrats with less canopy cover compared with the other two species. *Liopholis guthega* has been recorded in sub-alpine woodland where snow gum (*Eucalyptus pauciflora*) are present (Atkins, 2018). In the present study, *L. guthega* was found in sites with *E. pauciflora*, but the majority of individuals were collected away from canopy cover. Increased access to insolation away from tree canopies might be particularly important for *L. guthega*, which under current climatic conditions has the lowest number of days per season to feed, mate and give birth before temperatures drop, snow re-falls and torpor begins. Analysis revealed that *L. guthega* habitat tended to have smaller crevice widths, reflecting characteristics of granite boulders present at the site compared with larger rock slabs in *L. montana* and *L. whitii* sites. There was also less groundcover around *L. guthega* shelter sites. Patterns of interspecific differences in variables loading onto PC2 were less clear, but analysis captured some differences between *L. montana* and *L. whitii*, with a number of *L. montana* occurring in areas with high rock cover. There is a degree of variance not explained by the principal components analysis (29.9%), suggesting that other habitat variables not captured in this study might also be important between species.

The main habitat differences captured between *L. guthega* and its relatives were floristic or related to vegetation structure. This is to be expected given that *L. guthega* largely occupies the vegetatively distinct alpine zone. Under worst-case climate scenarios, the entire Australian alpine zone will be replaced by subalpine vegetation by 2050, reducing habitat above
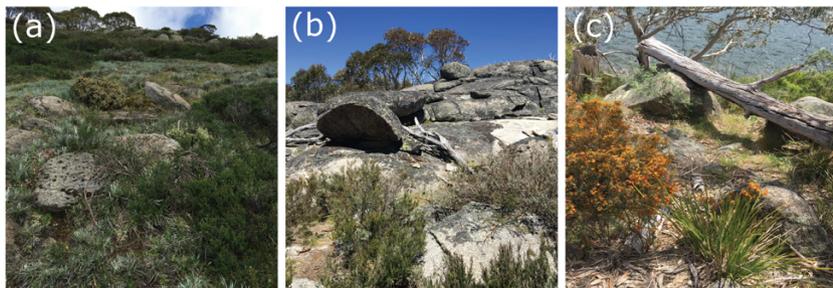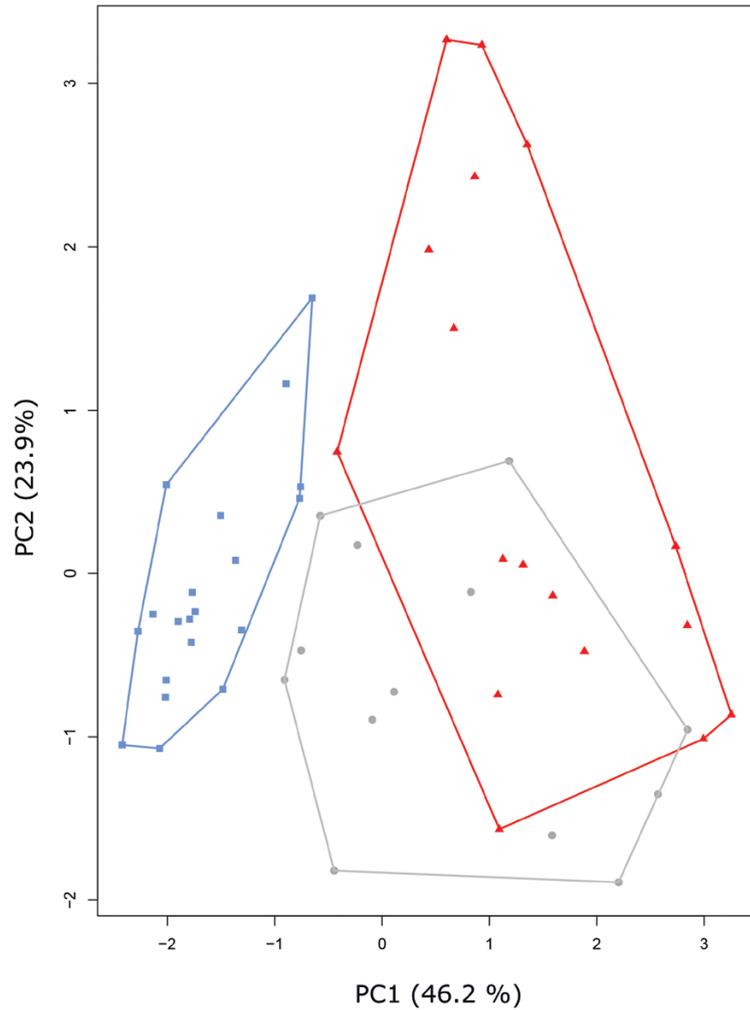
Figure 2. Scatter plot of first and second principal components (PC1 and PC2) of habitat traits. Traits were associated with the presence of trees, decreasing crevice width and ground cover (PC1, 46.2% of variance explained), and increasing rock cover, decreasing soil cover and mid-storey cover (PC2, 23.9% of variance explained). Symbols: blue squares, *Liopholis guthega* ($N = 20$); red triangles, *Liopholis montana* ($N = 17$); grey circles, *Liopholis whitii* ($N = 13$). Polygons enclose all individuals from each of the three species. Photographs show indicative habitat for *L. guthega* (A), *L. montana* (B) and *L. whitii* (C). All photographs were taken by A.F.S.

the treeline and altering seasonal snow cover (Green *et al.*, 1992; Hennessy *et al.*, 2003; Pickering, 2007). Additional changes in vegetation cover may occur from increased exotic plant invasions into higher elevation (Alexander *et al.*, 2016) and upland movement of feral and native herbivores (Hughes, 2003). Vegetative changes could lead to distribution shifts within the species group. At the observed zone of overlap between *L. guthega* and *L. montana*, the species were allopatric. Interspecific competition is a likely mechanism maintaining elevational partitioning in a range of taxa (Barve & Dhondt, 2017). Investigating competitive

behavioural differences in this species group will shed light on whether one species is likely to exclude another in instances of secondary contact.

Surprisingly little has been done to examine variability in CTmin with elevation (Spellerberg, 1972; Hertz & Nevo, 1981; Gvoždík & Castilla, 2001; Caldwell *et al.*, 2015; Huang & Tu, 2008b), particularly across the landscape scale, despite the theoretical prediction of lability in this trait. When CTmin was compared between populations, we found a notable intraspecific difference between *L. guthega* from the highest site (1860 m) and *L. guthega* from 1620 m. The high-elevation animals were able to tolerate colder temperatures than their congeners from lower
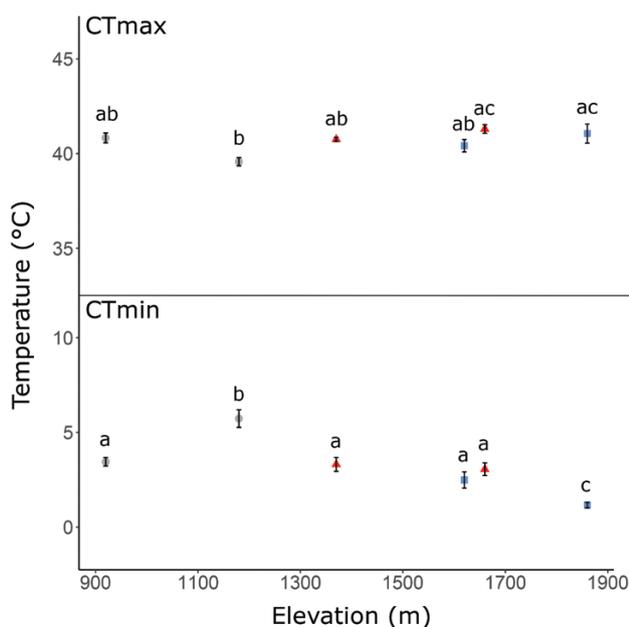

Figure 3. Mean ± SE critical thermal tolerance [maximum (CTmax) and minimum (CTmin)] for *Liopholis guthega* (blue squares), *Liopholis montana* (red trianges) and *Liopholis whitii* (grey circles). Elevation is in metres above sea level. Different letters denote statistical difference between populations.

elevations before losing the ability to right themselves. This suggests that cold alpine temperatures experienced at this elevation have driven a specific adaptation or a plastic response affecting CTmin. Low-elevation *L. guthega* were more similar in thermal tolerance to members of the other *Liopholis* species than to their high-elevation congeners, highlighting the lability of this trait within species across even small spatial scales. This population of *L. guthega* also had the widest tolerance breadth, probably in response to the range of temperatures experienced at high elevations. Thermal tolerance range tends to widen in animals experiencing more variability in the thermal environment (Gaston & Chown, 1999; Addo-Bediako *et al.*, 2000; Beck *et al.*, 2016).

Increases in body temperature from 15 to 30 °C resulted in variable differences in sprint ability, which are likely to be population specific, rather than directly linked to species or elevation. In general, most lizards ran faster between 15 and 25 °C, but fewer populations ran faster between 25 and 30 °C. Population differences in sprint speed often do not follow differences in critical thermal limits (Stork, 2012) and may be more closely linked to non-climate-related variables, such as predation pressure. At 15 °C, *L. guthega* from both populations were similarly slow compared with lowland species, suggesting that this alpine–subalpine species does not have a locomotive advantage at this temperature. This reflects field observations of all species, none of which were found outside their shelter sites at temperatures < 15 °C.

The thermal tolerance limits of *L. whitii* from the high-elevation population were very narrow and cannot be explained entirely by adaptive strategies to suit environmental conditions. The population had the lowest measured CTmax and was the least able to tolerate high temperatures. Correspondingly, sprint speed for this population did not change between heat treatments, in contrast to the overall pattern exhibited by *L. whitii* from 920 m and the other species. Temperature logger data suggested that maximal environmental temperatures were hotter at the high-elevation *L. whitii* site (during winter and
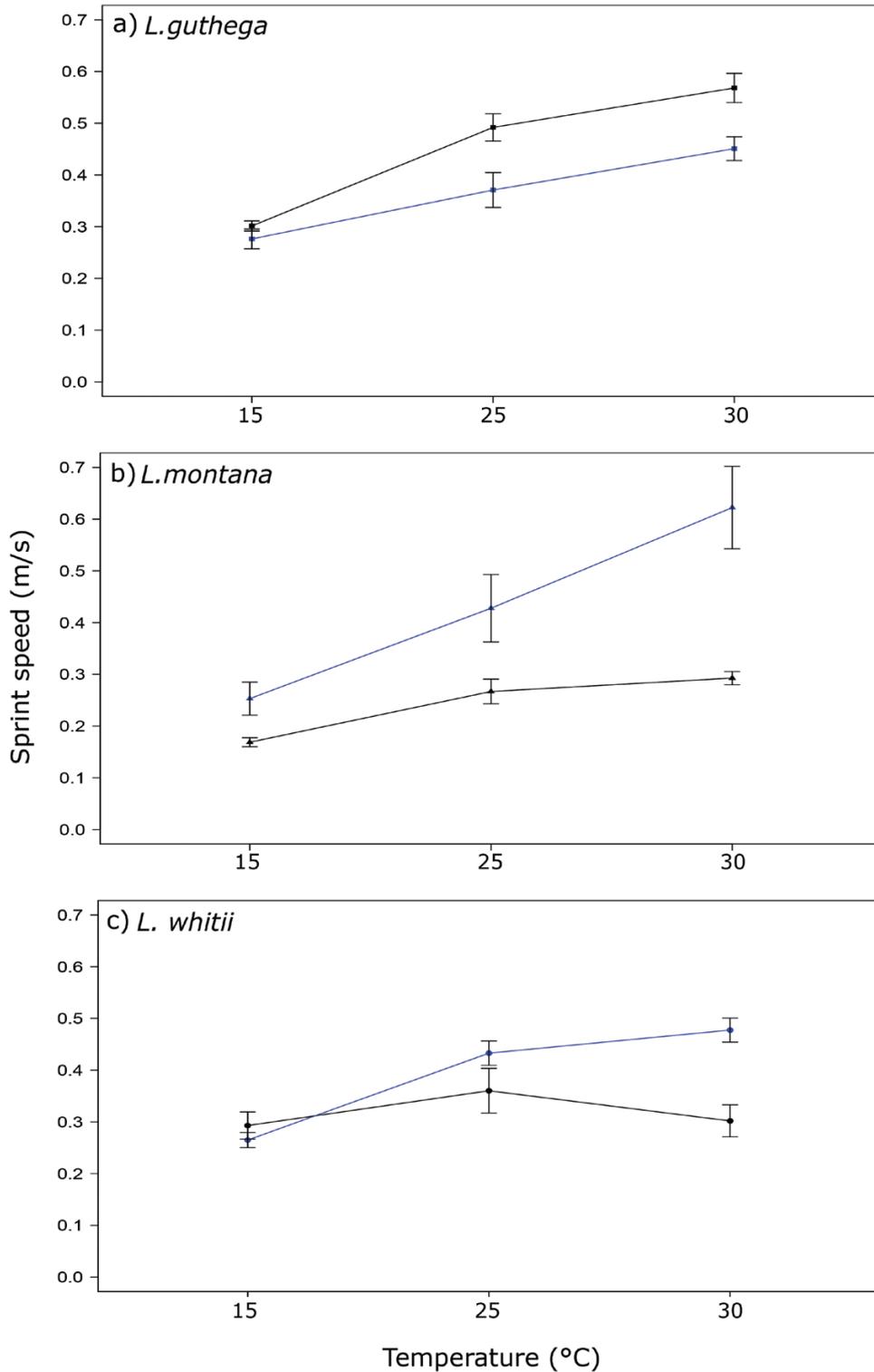
Table 3. ANCOVA comparing *Liopholis* population sprint speed at three ecologically relevant temperatures: 15, 25 and 30 °C

Source	SS	d.f.	MS	*F*-ratio	*P*-value
Site	2.39	5	0.48	10.65	< 0.001*
Treatment	9.50	2	4.75	106.05	< 0.001*
Sex	0.14	1	0.14	3.12	0.08
Site × treatment	2.35	10	0.23	5.24	< 0.001*
Snout–vent length	0.05	1	0.05	1.09	0.30

*Statistical significance ($P < 0.05$).

Figure 4. Maximal sprint speed in meters per second (mean ± SE) at three temperature treatments for high- (black) and low-elevation (dark blue) populations of *Liopholis guthega* (A), *Liopholis montana* (B) and *Liopholis whitii* (C).

spring) than at all other sites (Supporting Information, Table S3). We were unable to collect environmental temperature data during summer but suggest that the

site differences experienced during winter and spring might continue throughout the year. A low CTmax in response to high environmental temperatures

is initially a counterintuitive pattern, which might be explained by local environmental conditions. Likewise, Llewelyn *et al.* (2016, 2017) found a negative relationship between CTmax and environmental temperature in the skink species *Lampropholis coggeri*. Skinks from hotter environments tolerated lower temperatures than conspecifics from cooler environments and chose cooler microenvironments in which to thermoregulate (Llewellyn *et al.*, 2017). We might expect that high-elevation *L. whitii* are choosing cooler microenvironments within the hotter macro-environment and that their physiology has acclimatized to these lower temperatures (Llewellyn *et al.*, 2017). However, these animals were also least able to tolerate cold temperatures, with individuals from this population exhibiting a CTmin of between 4.4 and 9.1 °C. This was significantly higher than *L. whitii* from the lower population (2.0–5.2 °C). The minimal temperatures for this site in the month lizards were collected was 4.8 °C. Environmental temperatures at the site of the lower *L. whitii* population had consistently warmer minima. This pattern, or lack thereof, indicates some mismatch between critical thermal minima and environmental temperatures, potentially a result of non-adaptive evolutionary processes occurring in the high-elevation population, which is nearing the limit of the elevational extent of the species. Further investigation of microhabitat temperature variability at this site would strengthen our understanding of the conditions that might be influencing the narrow critical thermal limits of this *L. whitii* population.

Upper thermal limits were similar for all *Liopholis* populations except the high-elevation *L. whitii*. We suggest that this trait conservatism is the result of similar maximal environmental temperatures during the activity season, rather than the Bogert effect, where buffering of CTmax by behavioural thermoregulation could result in similar upper tolerances (Muñoz *et al.*, 2014). However, further environmental temperature data would strengthen this assertion. Studies investigating the effect of elevation on lizard CTmax have reported mixed results, with some intraspecific studies reporting decreases in CTmax (Hertz, 1979, 1981; Hertz *et al.*, 1979), whereas others have reported no pattern (Gvoždík & Castilla, 2001). In a review of lizard thermal trait variation, Clussela-Trullas & Chown (2014) highlighted the lack of consistency in trait responses. We suggest that spatial scale is an important consideration when making generalizations about the effect of elevation on lizard upper thermal limits. Other multispecies comparisons reporting differences in CTmax were conducted over much wider geographical scales than the present study (Huang & Tu, 2008a; Caldwell *et al.*, 2015).

The effects of warming temperatures under future climate scenarios have been modelled for *L. guthega* (Atkins, 2018). It was found that the species will reach minimal activity thresholds more frequently and experience a reduced torpor period, resulting in an increase in potential activity time (Atkins, 2018). Comparable upper thermal limits in *L. guthega* across its elevational extent found in the present study support the idea that this species as a whole might experience positive effects of climate warming. The adaptive capacity of the species will also be determined by their capacity to adjust feeding, thermoregulatory and competitive behaviour in response to environmental change (Kearney *et al.*, 2009; Atkins *et al.* 2017; Caldwell *et al.*, 2017; Camacho *et al.*, 2018).

CONCLUSIONS

Trait comparisons in the *Liopholis* group have revealed a pattern of thermal tolerances that is likely to be the result of localized adaptation, physiological plasticity, or both. We suggest that the elevation replacement distribution in this group might be influenced somewhat by thermal tolerance constraints, but that behavioural and small-scale habitat differences might also play a role. Like Caldwell *et al.* (2015), we cannot identify whether patterns in thermal tolerances are the result of heritable genetic differences or plasticity in response to environmental conditions. We share the view that common garden experiments would be valuable in teasing out these effects but acknowledge the challenge of conducting such work on rare species with conservation status, such as those in the present study. More broadly, our findings are important in understanding the pattern of species distributions and variability in thermal tolerances between species, but also within species across small geographical scales.

AUTHOR CONTRIBUTIONS

A.F.S. carried out fieldwork, laboratory experiments and data analysis and led the design of the study and the writing of the manuscript. B.B.M.W., D.G.C., N.C. and Z.S.A. contributed to the design of the study and drafting of the manuscript. M.S., M.M.G. and G.M.W. contributed to the drafting of the manuscript. All authors gave final approval for publication.

ETHICAL STATEMENT

Research was conducted in accordance with appropriate collection and research permits (New South Wales: SL101798) and was approved by the Monash University animal welfare committee (BSCI/2016/24).

CONFLICT OF INTEREST

We have no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Adjusted *P*-value of the post hoc pairwise multiple comparisons procedure (Tukey's HSD tests) applied to the one-way ANCOVA for comparisons of critical thermal limits (CTmin and CTmax) and tolerance breadth (TTB) in *Liopholis guthega* (1860 and 1620 m), *Liopholis montana* (1660 and 1370 m) and *Liopholis whitii* (1180 and 920 m). Statistical significance ($P < 0.05$) is highlighted in bold.

Table S2. Adjusted *P*-value of the post hoc pairwise multiple comparisons procedure (least squared means comparisons) applied to the two-way repeated-measures ANCOVA for comparisons of sprint speed at three ecologically relevant temperatures in *Liopholis guthega* (1860 and 1620m), *Liopholis montana* (1660 and 1370 m) and *Liopholis whitii* (1180 and 920 m). Statistical significance ($P < 0.05$) is highlighted in bold.

Table S3. Monthly environmental temperatures (in degrees Celsius) as measured by Maxim Thermochron iButtons during winter and spring at different elevations (in metres above sea level). Sites covered in snow show only a small difference or no difference in maximal and minimal temperatures and are highlighted in bold. iButtons at 1180 and 920 m failed in November, and data could not be retrieved.