**RESEARCH PAPER**

# The evolution of viviparity opens opportunities for lizard radiation but drives it into a climatic cul-de-sac

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## ABSTRACT

**Aim** Evolutionary radiations into novel areas or niches require innovative adaptations. However, rapid subsequent changes in these novel conditions might demand rapid re-adaptations to secure population persistence and prevent extinction. We propose that reptilian viviparity (live birth) is consistent with such a scenario. Using the *Liolaemus* lizard radiation, we investigate the hypotheses that historical invasions of cold climates have been permitted by transitions to viviparity, and that this parity mode is irreversible. Then, we investigate whether these combined factors restrict viviparous lizards to cold climates, and hence, whether viviparous species are particularly threatened by climate change.

**Location** South America.

**Methods** We employ phylogenetic analyses to investigate evolutionary transitions in reproductive modes and their consequences for environmental restrictions in viviparous lizards. We then employ climatic projections to predict the impact of climate change on the future persistence of these organisms.

**Results** The oviparity-to-viviparity transition is consistently associated with colonization of cold climates, and appears to be irreversible. Since viviparity seems less viable (compared with oviparity) in warm climates, species that evolve viviparity in cold climates are likely to remain adaptively constrained to such environments. Therefore, upward–poleward advances of climate warming will cause severe shifts and contractions of viviparous species ranges, threatening major extinctions over the next half century.

**Main conclusions** Viviparity has been largely responsible for the successful radiation of *Liolaemus* into cold climates, but since this adaptation is predominantly viable in these environments and is unlikely to re-evolve into oviparity, viviparity may prove to be an evolutionary dead-end for lizards facing rapid climate change.

**Keywords**

**Adaptive radiation, climate change, climatic envelope, extinction, *Liolaemus*, lizards, South America, viviparity.**

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## INTRODUCTION

The evolution of adaptive innovations facilitates the radiation of organisms into previously unexploited selective environments, such as unoccupied areas or niche dimensions (Losos, 2010). The genetic potential of lineages to repeatedly evolve such innovations results in multiple independent opportunities for adaptive expansions across vast spatial or temporal scales. Among squamate reptiles (lizards and snakes), for whom reproduction is heavily temperature dependent, the evolutionary lability of parity modes has been essential to overcome the selective stress imposed by reduced environmental temperatures on reproduction during radiations into colder climates (Guillette, 1993; Shine, 2005). In these organisms, evolutionary transitions to viviparity from oviparity have mostly occurred in species at high latitudes and elevations, where temperatures are lower

(Blackburn, 2000; Shine, 2005). Therefore it has been suggested that the fitness costs imposed by low and unstable environmental temperatures on external incubation of eggs are the primary force underlying the evolution of viviparity in cold climate squamates (Shine, 2005).

The traditional explanation for the evolution of viviparity, the 'cold climate hypothesis', posits that low temperatures experienced in cold climates by externally developing eggs, compromise or preclude successful incubations. Therefore, selection favours the evolution of prolonged retention of eggs within the female, providing a warmer and more efficient incubation environment stabilized by the female's thermoregulation (Blackburn, 2000; Shine, 2005). An alternative, the 'maternal manipulation hypothesis', predicts that egg retention results in higher offspring viability through maternal effects (Shine, 1995; Shine, 2005). For example, recent studies have shown that viviparity in lizards enhances the ecological performance of hatchlings via modification of fitness-relevant traits (Shine, 1995; Ji *et al.*, 2007). Similarly, the stable (rather than high) temperatures provided by females to their retained eggs contribute to these developmental benefits (Shine, 2004; Shine, 2005). Finally, the prevalence of viviparity in cold climates is thought to be associated with the high ecological and reproductive costs (e.g. from pregnancy burden, lower reproductive frequency) it imposes in warm climates (Shine, 2005). These costs appear to explain the fact that viviparity rarely occurs in such climates (about 200 species, about 17% of viviparous lizards in total) despite its demonstrated benefits for offspring fitness (Shine, 2004; Shine, 2005).

Collectively, research on reptilian parity modes suggests that the remarkable evolutionary lability of the oviparity–viviparity transition is likely to have played a central role in the success of radiations into cold areas globally. However, while this adaptability may have contributed to the historical evolutionary success of squamate reptiles, it may also be partly responsible for the increasing global extinction risk these organisms face as a result of climate change. Evolutionarily, a major problem of viviparity is that transitions back from viviparity to oviparity appear very unlikely (Lee & Shine, 1998; Shine & Lee, 1999; Shine, 2005). Recent evidence suggests only one clear case of reversal in boas (Lynch & Wagner, 2010), and phylogenetically equivocal evidence for two cases of reversals in vipers (Fenwick *et al.*, 2012). Therefore, poleward and upward displacements of thermal limits for viviparity tolerance, caused by climate warming, may progressively restrict the range of viviparous species until they collapse to extinction. Additionally, the negative impacts of range restrictions may be compounded by competitive invasions of oviparous species from warmer environments following these thermal displacements (Huey *et al.*, 2010; Sinervo *et al.*, 2010; Thomas, 2010). Given that climate warming is currently occurring globally (Hulme, 2005; IPCC, 2007) and distorting species ranges and dispersal (Thomas *et al.*, 2006), this is a matter of serious concern (Hewitt & Nichols, 2005; Thomas, 2005; Parmesan, 2006). Indeed, evidence suggests that climate change is posing serious risks of extinction to lizards globally, and that the rates and risks of extinction are considerably higher for viviparous species (Sinervo *et al.*, 2010). Hence, the failure of these organisms to evolve oviparity from viviparity (Shine, 2005), or to quickly adapt in other ways to rapid warming (Chevin *et al.*, 2010; Huey *et al.*, 2010; Sinervo *et al.*, 2010), seems to impose a major risk of short-term and large-scale extinction for cold climate viviparous species. However, the reasons why viviparous species may experience higher risks of extinction remain to be explained.

Here, we investigate the evolution of viviparity in the *Liolaemus* lizard adaptive radiation and predict its future impact under scenarios of climate warming. These South American iguanians offer a unique model system. *Liolaemus*, one of the most species-rich living amniote genera, consists of 220+ species adapted to perhaps the widest diversity of climatic conditions known among lizards (Pincheira-Donoso *et al.*, 2008b), occurring from the Atacama Desert to Tierra del Fuego in Patagonia (*c.* 54° S, the southernmost area inhabited by lizards), and from sea level to over 5000 m in the Andes (Pincheira-Donoso *et al.*, 2008a). Additionally, viviparity has been shown to have evolved several times independently (Schulte *et al.*, 2000). We investigate whether the evolution of viviparity is linked to the colonization of cold climates in this lineage, and whether oviparous and viviparous species are adaptively segregated in different climatic conditions where either parity mode is more viable. The primary prediction, based on the high fitness costs of viviparity in warm climates and the reported genetic irreversibility of this parity mode in lizards, is that viviparous species are more strongly restricted by latitudes and elevations (and hence, by cold climates), and have narrower climatic niches, than oviparous species. We investigate phylogenetically the irreversibility of the oviparity–viviparity transition. Finally, we perform projections of the South American climate into the future scenarios suggested by the Intergovernmental Panel on Climate Change (IPCC, 2007), and show that the latitudinal/elevational regression line, describing environmental range limits of viviparous species, will move poleward and upward, causing severe restrictions and fragmentation of the currently available biogeographic range of *Liolaemus* species.

## MATERIALS AND METHODS

### Data

We studied 153 *Liolaemus* species spanning the entire supraspecies phylogenetic, ecological and geographical diversity known for this clade. We collected data for reproductive modes, and latitudinal and elevational distributions. Reproductive mode data were organized into two states of the response variable, oviparity for egg-laying species and viviparity for live-bearing species (Blackburn, 2000; Shine, 2005). Data come from multiple published sources where reproductive modes are reported for *Liolaemus* species (Schulte *et al.*, 2000; Pincheira-Donoso *et al.*, 2008b; and references in Table S1 in Supporting Information), and from direct observation of females. For two species (*Liolaemus fitzingerii* and *Liolaemus bibronii*) we found conflicting reports of parity modes (Donoso-Barros, 1966; Cei, 1986).

In both cases, we used the parity mode obtained by direct observation of specimens (J. M. Cei, pers. comm.). All species for which no observational data on parity modes were available (e.g. speculations of oviparity or viviparity) were excluded from the analyses. These data have been summarized in a single study (Pincheira-Donoso et al., 2008b), where complete details of the published sources per species are presented.

Data on the geographical distribution of Liolaemus species were obtained from the above-mentioned sources, from additional studies covering several species (Cruz et al., 2005; Pincheira-Donoso et al., 2007, 2008a,b; Pincheira-Donoso, 2011; Pincheira-Donoso & Tregenza, 2011), from 8500+ individual records personally obtained by D.P.-D. from several collections around the world (see Acknowledgements) and from extensive field work in the countries where Liolaemus occur. As stated above, viviparity has consistently evolved at high latitudes and elevations (Blackburn, 2000; Schulte et al., 2000; Shine, 2005). Therefore, high elevations at low latitudes can be considered equivalent to low elevations at high latitudes (Lutgens & Tarbuck, 1998). Hence, we analysed our data using information for both latitudinal and elevational distribution for each species. Depending on the analysis, we used both latitude and elevation as separate variables in the same test, or combined them into a single variable that incorporates the effect of both, known as the adjusted latitudinal midpoint (ALM) per species. This combined scale has been employed in several comparative studies investigating the effect of climatic variation on adaptations in Liolaemus species (Cruz et al., 2005; Pincheira-Donoso et al., 2007, 2008a; Pincheira-Donoso & Tregenza, 2011). The ALM is calculated on the assumption that environmental temperature in altitudinal transects declines by 0.65 °C for each 100 m of increased elevation (Lutgens & Tarbuck, 1998; Cruz et al., 2005). Cruz et al. (2005) obtained a corrected latitudinal value for latitude and altitudinal thermal covariation using the formula

$$\text{ALM} = 0.009x - 6.2627 + y \qquad (1)$$

where $x$ is the altitudinal midpoint per species and $y$ the latitudinal midpoint. Equation (1) returns ALM values for each species (Cruz et al., 2005).

## Statistical analyses and phylogenetic control

Studying the evolution of viviparity requires a phylogenetic context to investigate the adaptive lability of this life-history transition (Shine, 2005). Therefore, we employed a phylogenetic hypothesis for Liolaemus lizards derived from Espinoza et al. (2004) and Abdala (2007) (Fig. 1). Phylogenetic studies within Liolaemus have consistently shown the existence of a major monophyletic clade nested within the genus, characterized by a patch of enlarged scales on the thigh, termed the boulengeri complex (e.g. Schulte et al., 2000; Espinoza et al., 2004; Abdala, 2007). We used the topology of Espinoza et al. (2004) as the basis for our Liolaemus phylogeny, but replaced the monophyletic boulengeri complex with Abdala's (2007) phylogeny for this clade, as it contains a large number of species sampled in our

dataset. This resulted in a well-supported phylogenetic hypothesis for 84 species (Fig. 1). Our analyses are based on this single phylogenetic hypothesis because we lack the combined molecular data to infer a comprehensive phylogeny for all the species in our dataset.

We first tested the hypothesis that viviparity has evolved from oviparity during colonization of cold environments. We analysed parity mode as a binary response variable (0 for oviparity, 1 for viviparity). We then conducted phylogenetic logistic regressions with reproductive mode as the binary response variable and ALM as the predictor using the PlogReg.m code implemented for MatLab (Ives & Garland, 2010). This logistic regression deals with categorical dependent variables, thus preventing the use of dependent traits as dummy independent variables. For phylogenetic logistic regressions we employed a phylogeny formatted as a variance–covariance matrix, $\mathbf{W}$ (Ives & Garland, 2010). Since the sources for our phylogeny derive from both molecular and morphological data (Espinoza et al., 2004; Abdala, 2007), it has branch lengths equal to 1.0. Therefore, prior to the transformation of the tree into a matrix we scaled branches to make the tree ultrametric using the cladogram transform in the software FigTree (Rambaut, 2009). Phylogenetic logistic regressions use the Firth correction (Firth, 1993) to reduce the bias in logistic regression coefficients introduced by maximum likelihood estimates (Ives & Garland, 2010). Subsequently, the code performs a parametric bootstrapping by simulating 2000 datasets to obtain bootstrap means and bootstrap confidence intervals, and to test the hypothesis that viviparous species are more likely to evolve in colder climates once phylogenetic relationships are taken into account.

We then investigated whether transitions in parity modes are mostly unidirectional from oviparity to viviparity (Dollo's law), as predominantly observed among squamates (Lee & Shine, 1998; Shine & Lee, 1999). We performed likelihood reconstructions of character evolution with Mesquite v.2.01 (Maddison & Maddison, 2011), to test for irreversibility of viviparity. We employed a binary-state speciation and extinction (BiSSE) model that includes transition $q$ (where $q_{01}$ is forward transition and $q_{10}$ is backward transition), speciation $\lambda$ and extinction $\mu$ rate parameters, implemented in the Diverse package of Mesquite (Maddison et al., 2007; Goldberg & Igic, 2008). These parameters ($k$) were directly estimated from the data. To investigate transitions of parity modes we compared four BiSSE models, with six ($q_{01}, q_{10}, \lambda_0, \lambda_1, \mu_0, \mu_1$) and four ($q_{01}, q_{10}, \lambda_0 = \lambda_1, \mu_0 = \mu_1$) parameters where parity mode is assumed to be reversible, and with five ($q_{01}, \lambda_0, \lambda_1, \mu_0, \mu_1$) and three ($q_{01}, \lambda_0 = \lambda_1, \mu_0 = \mu_1$) parameters where viviparity is assumed to be irreversible (i.e. backward transition rate $q_{10} = 0$). For these two irreversible models of trait evolution to exhibit both character states in the tips, a tree root fixed to character state 0 (i.e. oviparity) is required. Therefore, we fixed the tree root to an oviparous state, using a modified version of the Diverse package of Mesquite (v.2.01) developed by R. G. FitzJohn (Goldberg & Igic, 2008). We then applied a model selection approach based on the Akaike information criterion (AIC) (Burnham & Anderson, 2002).
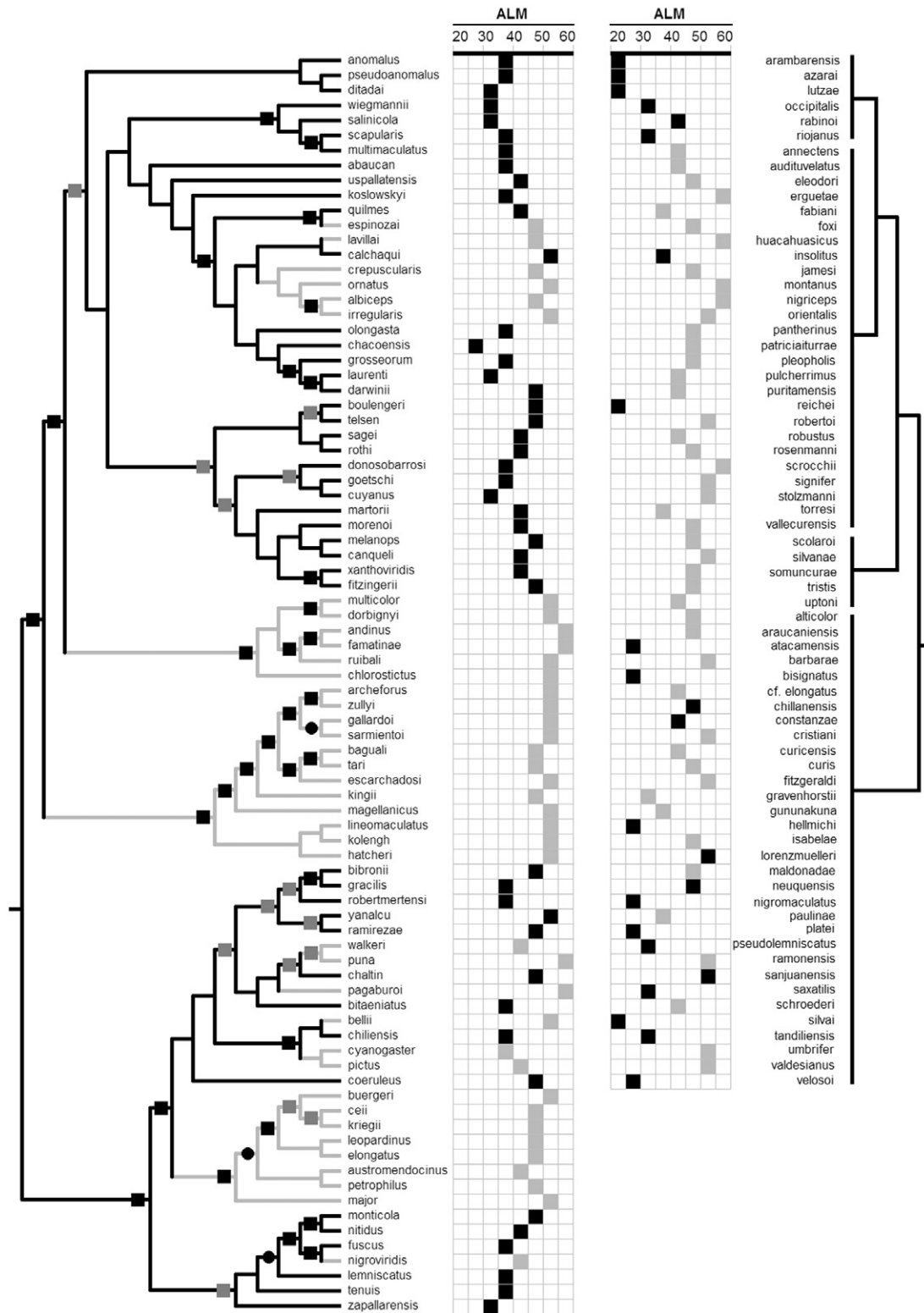
**Figure 1** Phylogenetic relationships within the genus *Liolaemus* and the distribution of oviparity (black terminal branches and cells) and viviparity (grey branches and cells) across a climatic gradient represented by the adjusted latitudinal midpoint (ALM; see text for details). The resolved phylogeny (left) was taken from the literature (see Materials and Methods) and has been used for phylogenetic analyses. Squares at nodes indicate bootstrap support (black, 90–100%; grey, 70–89%) and circles indicate Bayesian posterior probabilities of ≥ 90% (taken from the original sources; see Materials and Methods). The phylogeny on the right includes the remaining *Liolaemus* species for which reproductive data are available but which have not been included in the main phylogeny. Therefore, we present a tree resolved to the level of main subclades based on previously validated traits that distinguish among them.

We then addressed the question of whether oviparous and viviparous species are climatically segregated into primarily warm and cold environments, respectively. We created a bivariate climatic space with axes of increasing latitude (on *x*) and increasing elevation (on *y*) (Fig. 3), based on the maximum limits of latitude and elevation known for each oviparous species, and on the minimum limits of latitude and elevation per viviparous species. This approach is an idealization of a geographical map where species are placed based at the limits of their distributions in latitude and elevation on this bivariate climatic-space plot. We used standardized major axis (SMA) regressions to find the best-fit linear combination of latitude and altitude that best described the upper limits of oviparous species ranges and the lower limits of viviparous species ranges. SMA was preferred over ordinary least squares, because the former considers measurement error to exist on both *x*- and *y*-axes, while the latter assumes the explanatory variable to be fixed. SMA also allowed us to ask whether the mean upper limit regression line for oviparous species shared a common slope and intercept with the mean lower limit regression line for viviparous species. We then tested whether viviparous and oviparous species range limits tended to occur in different mixtures of altitude and latitude, by asking whether the two groups were 'shifted' along the common regression line. Further, we tested whether the SMA regression lines differed significantly from Cruz *et al.*'s (2005) regression line (equation 1). Finally, we tested differences in the bioclimatic flexibility of the two parity modes by extracting the perpendicular residuals from a common SMA regression line, and using a variance ratio test to ask whether the upper altitudinal/latitudinal limit of oviparous species had greater variance than the lower limit of viviparous species. Analyses were performed using the major axis regression package 'smatr' (Taskinen & Warton, 2011) in R v.2.12.0 (R Development Core Team, 2010). We confirmed that the conclusions of the standardized major axis regressions were not biased by evolutionary history, using the phylogenetic reduced major axis regression function in the R library 'phytools' (Revell, 2012).

To examine the potential impacts of climate warming on the future range limits of viviparous species, we mapped the current latitudinal and altitudinal range limits derived from our SMA regression line of ALM. We then superimposed regression lines of mean environmental temperatures from 1979–2011, sourced from the ERA-Interim dataset from the European Centre for Medium Range Weather Forecasts (ECMWF). We found an excellent match between ALM-derived range limits and the 7 °C regression line for April–June, which describe a lower limit to autumn environmental temperature for the persistence of viviparous species. We then projected this 7 °C Apr–June environmental temperature regression line forward to the year 2060 into a climate change scenario for Central and South America, published by the Intergovernmental Panel on Climate Change (Magrin *et al.*, 2007) and derived from the UK Met Office Hadley Centre Global Environmental Model 1 based on moderate carbon emissions (A1B; Fig. 4b).
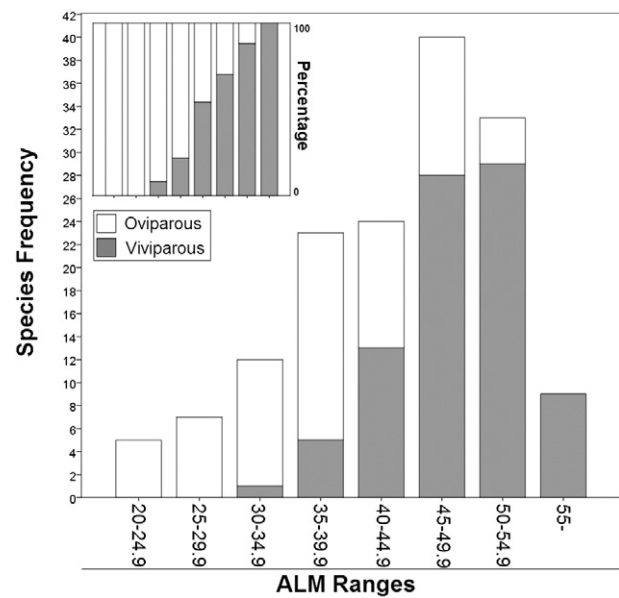


**Figure 2** Climatic segregation of oviparous and viviparous *Liolaemus* species. Frequency distribution of both parity modes across a spatial and climatic gradient represented by the adjusted latitudinal midpoint (ALM) (higher ALM, higher latitude–elevation) in absolute numbers (main plot) and scaled for percentages (small plot inside).

## RESULTS

Species with different reproductive modes are segregated in different climatic areas ($F_{1,151} = 95.26$, $P < 0.001$; Figs 1 & 2). Oviparous species occupy primarily warm environments [mean $ALM_{oviparous} = 37.91 \pm 8.3$ (SD), range = 22.76–54.18], while viviparous species inhabit colder climates (mean $ALM_{viviparous} = 48.69 \pm 5.3$, range = 33.50–60.04; Fig. 2). Phylogenetic logistic regressions confirmed that viviparity is likely to have evolved as an adaptive response to radiations into colder climates (high latitudes–elevations), as increases in the ALM distribution of species predict a significantly higher probability of being viviparous (Table 1). Parametric bootstrapping revealed qualitatively identical results, with the viviparous parity mode being significantly more likely to be found at higher latitudes–elevations (Table 1). Our phylogenetic likelihood analyses of character evolution support the model of irreversible viviparity compared with rival models (Table 2). The 'most likely' model of parity mode evolution describes irreversible transition. The paired 'equal rates' model, with reversible parity mode, had ΔAIC from this best model of 2.65 units, making it 0.266 times as likely to minimize information loss (Table 2).

The lower latitude–altitude limit of viviparous species lay tightly along a linear regression (standardized major axis regression, altitudinal limit = $6022 - 125.5 \times$ latitudinal limit, $R^2 = 0.69$, $P < 0.001$; Fig. 3), while the upper limit of oviparous species lay loosely along a near-significant regression line (altitudinal limit = $6845 - 150 \times$ latitudinal limit, $R^2 = 0.05$, $P = 0.059$; Fig. 3). Despite these differences, the upper limit regres-

**Table 1** Results of phylogenetic logistic regression parameter estimates with Firth correction for the effect of adjusted latitudinal midpoint (ALM) on the evolution of binary parity modes (0 = oviparity, 1 = viviparous) in *Liolaemus* lizards. Both logistic regression and bootstrap analyses based on simulations suggest that viviparity has independently evolved in several evolutionary events during the radiation of these lizards into colder environments. The phylogenetic signal (*a*) on the expression of parity mode is small and non-significant.

| Parameter* | Estimate | SE† | *t*-score | *P*-value | Bootstrap mean‡ | Bootstrap confidence interval‡ | Bootstrap *P*-value‡ |
|---|---|---|---|---|---|---|---|
| *a* | −0.29 | – | – | – | −3.05 | (−3.99, −1.10) | 0.46 |
| $b_0$ (intercept) | −0.31 | 0.58 | −0.53 | 0.59 | −0.30 | (−0.88, 0.24) | 0.29 |
| $b_1$ (ALM) | 1.65 | 0.38 | 4.34 | < 0.001 | 1.65 | (1.04, 2.46) | < 0.001 |

*The dependent variable was standardized to have mean 0 and variance 1 prior to analysis.
†Standard errors (SE) of the estimates and confidence intervals were calculated using the generalized estimating equations approximation (see equation 9 in Ives & Garland 2010).
‡Parametric bootstrapping was performed by simulating 2000 datasets using the MatLab code PlogReg.m to obtain bootstrap means and confidence intervals. The same parametric bootstrapping was employed to test the null hypothesis that there is no phylogenetic signal in the residuals and that the regression coefficient equals 0 (see Ives & Garland, 2010, for details).

**Table 2** Model comparison tests of irreversibility in parity modes for *Liolaemus* lizards based on binary-state speciation and extinction (BiSSE) models of character evolution with trait transition (*q*), speciation ($\lambda$) and extinction ($\mu$) rate parameters estimated for oviparity (0) and viviparity (1) states. Four BiSSE models are compared, two where diversification is character-independent ($\lambda_0 = \lambda_1$, $\mu_0 = \mu_1$), and two where diversification is character-dependent ($\lambda_0$, $\lambda_1$, $\mu_0$, $\mu_1$). Within each pair of models we assume a reversible ($q_{01}$, $q_{10}$) and an irreversible ($q_{01}$, $q_{10} = 0$) character trait transition. The likelihood of each model (ln*L*), and their corresponding ΔAIC (AIC, Akaike information criterion) are reported. AIC-based analyses of model selection supports the irreversible character-independent diversification model ($q_{01}$, $q_{10} = 0$, $\lambda_0 = \lambda_1$, $\mu_0 = \mu_1$), in bold.

| Model | $\lambda_0$ | $\lambda_1$ | $\mu_0$ | $\mu_1$ | $q_{01}$ | $q_{10}$ | ln*L* | ΔAIC |
|---|---|---|---|---|---|---|---|---|
| Stationary | 0.262 | | $1.4 \times 10^{-6}$ | | 0.048 | 0.041 | −228.965 | 2.65 |
| **Fixed** | **0.262** | | **$9.5 \times 10^{-6}$** | | **0.053** | **0** | **−228.641** | **0.00** |
| Stationary | 0.248 | 0.284 | $1.3 \times 10^{-5}$ | $8.1 \times 10^{-6}$ | 0.046 | 0.049 | −228.826 | 6.37 |
| Fixed | 0.253 | 0.274 | $2.4 \times 10^{-6}$ | $9.7 \times 10^{-5}$ | 0.053 | 0 | −228.554 | 3.83 |

sion line for oviparous species and the lower limit regression line for viviparous species did not differ significantly in either slope or intercept (slope test, $\chi^2_1 = 1.754$, $P = 0.19$; intercept test, $\chi^2_1 < 0.001$, $P = 0.99$). However, oviparous upper limits and viviparous lower limits tended to occupy different zones of the common regression line (test of shift along common axis, $\chi^2_1 = 5.59$, $P = 0.02$; Fig. 3), with oviparous species upper ALM-distributional limits tending to occur at high latitudes rather than high elevations. The best-fit range-limit regression line, common to oviparous and viviparous species (altitudinal limit = $6251 - 133 \times$ latitudinal limit, $R^2 = 0.42$, $P < 0.001$), differs significantly from Cruz *et al.*'s (2005) formula for adjusted latitudinal midpoints ($r_{151} = 0.23$, $P < 0.01$), suggesting that range extremes cannot be described perfectly by this adjustment formula. However, Fig. 3 shows that the difference between SMA regression lines and Cruz *et al.*'s regression line is rather small, albeit significant. Reduced major axis regressions with phylogenetic control, with slopes tested against the SMA regression lines reported above, confirmed that the derived regression lines were not biased by evolutionary history (comparison of regression line slope common to viviparous and oviparous species, $t_{80.12} = 1.11$, $P = 0.27$; comparison of viviparous regression lines,

$t_{34.13} = 1.03$, $P = 0.31$; comparison of oviparous regression lines, $t_{42.37} = 0.42$, $P = 0.68$).

Scatter around the shared regression line of altitude and latitude was much greater among the upper environmental limits of oviparous species than among the lower environmental limits of viviparous species (variance ratio test, $F_{67,84} = 2.61$, $P < 0.001$; Fig. 3). Therefore, despite having a common mean range limit regression line, oviparous species are less tightly packed around the regression line, showing greater variance in range limit than viviparous species and, when studied in isolation, only a near significant regression line. Rearranging the mean range limit regression line shows that, on average, viviparous species persist if (latitude + $0.0075 \times$ altitude) > 47.

The current range of suitable environmental conditions for viviparity spans the full length of the Andes area inhabited by *Liolaemus* (from *c.* 10° S) and covers all of Patagonia (Fig. 4a). The reduction in suitable bioclimatic range caused by the climate warming scenario describing moderate carbon emissions (Fig. 4b) results in large-scale territorial losses (relative to current suitable viviparity area in 'no change scenario', Fig. 4a). The bioclimatic envelope of persistence for viviparous species is dramatically reduced and fragmented, showing clear evidence of
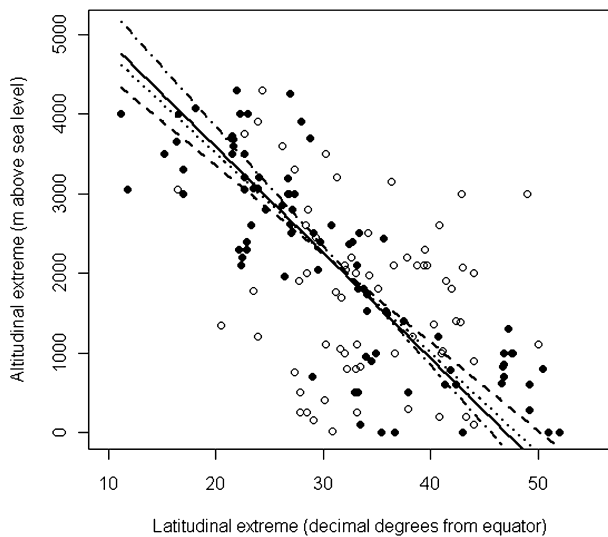
**Figure 3** Analyses on a bivariate climatic map based on increasing latitude *x* and elevation *y* showing the upper distributional limits of oviparous (open circles) and lower limits of viviparous (black circles) species of *Liolaemus*. Lines include the 'shared' major axis regression of lower viviparous and upper oviparous distributional limits (solid line), and a major axis regression derived from Cruz *et al.*'s (2005) equation (dashed line). Dotted and dot-dash are the best fit major axis regression lines for upper limits of oviparous species, and lower limits of viviparous species, respectively.
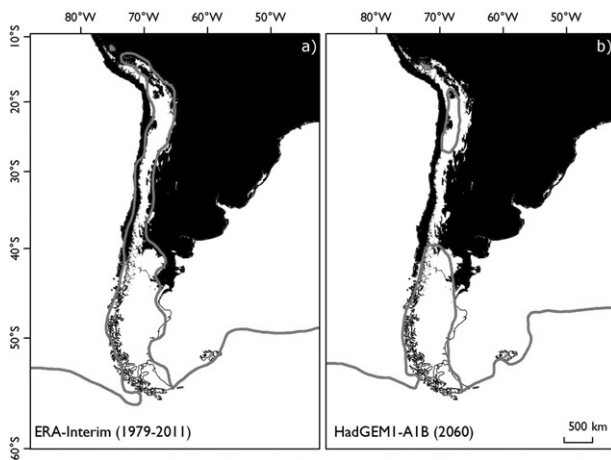


**Figure 4** Projection of the 'moderate emissions' climatic scenarios for South America, superimposed on the adjusted latitudinal regression line that delimits the current boundary between oviparous (black area) and viviparous (white area) *Liolaemus* species today. (a) The projected 7 °C mean temperature regression line for April–June, averaged over 1979–2011 (grey) matches well the current distributional boundary of oviparous and viviparous species. (b) The projected 7 °C mean temperature regression line for April–June in 2060, assuming moderate $CO_2$ emissions, dramatically reduces and fragments the bioclimatic envelope describing the range limits of viviparous species.

a north–south divide in viviparous range limits, with implications for species persistence and dispersal. We expect the magnitude of range loss and fragmentation to be even more severe under scenarios of higher carbon emissions.

## DISCUSSION

Our study shows that the evolution of viviparity in *Liolaemus* is associated with radiations into cold climates, where this parity mode appears to be adaptively confined. This is consistent with the hypothesis that cold and unstable climates impose strong selection on squamate reproduction, promoting the evolution of viviparity (Shine, 2005). In these climates, female behavioural thermoregulation provides the conditions to successfully complete embryo development within the female (Shine, 2004, 2005). Oviparity, in contrast, predominates in warm climates where egg incubation is possible. However, while distributions of viviparous species are strongly restricted to cold climates, the distributions of oviparous species are more widely climatically dispersed. Interestingly, oviparous species are more likely to expand into high latitudes than into high elevations (Fig. 3), possibly due to the impact of highly fluctuating environmental conditions in high mountains like the Andes (Nagy & Grabherr, 2009). Projections of climate change predict that under persistent warming, and based on evidence for irreversible parity mode, viviparous *Liolaemus* species will experience severe range contractions, with a remarkable north–south fragmentation of their suitable climatic areas, followed by major extinction events within the next few decades.

### The evolution of viviparity and the *Liolaemus* radiation

*Liolaemus* is one of the most successful vertebrate radiations (Pincheira-Donoso *et al.*, 2008b). Cold climates (the Andes and Patagonia) have been extensively colonized by these lizards, where several vertebrate communities are dominated by *Liolaemus* species (Cei, 1986; Schulte *et al.*, 2000; Pincheira-Donoso *et al.*, 2008b). The evolution of viviparity appears to be a major adaptive strategy underlying their successful invasion of such climates (Schulte *et al.*, 2000). The view that viviparity in *Liolaemus* has been linked to the invasion of cold climates is supported by previous evidence that this lizard radiation seems to have occurred in parallel with the uplift of the Andes at least 20 Ma (Schulte *et al.*, 2000; Albino, 2008), while the alternative hypothesis for an Andean origin has been rejected (Schulte *et al.*, 2000). Indeed, in this and other work (Pincheira-Donoso *et al.*, 2008b), it has been shown that about 55% of *Liolaemus* species (where parity mode is known) are viviparous. Our phylogenetic logistic regression supports this view. Almost all *Liolaemus* species that have successfully invaded extreme cold areas in the Andes and Patagonia are viviparous, while lower-elevation species are almost entirely oviparous (Fig. 2). However, our bioclimatic regression analysis reveals that oviparous upper limits are dominated by latitude rather than by elevation. Therefore, oviparous lizards are more likely to enter colder environments in

Patagonia (high latitudes) than in the Andes (high elevations). An explanation for this finding may be that high elevations in the Andes are more climatically unstable (Nagy & Grabherr, 2009), which may impose stronger detrimental effects on external egg incubation (see above).

The evolution of viviparity predominantly in cold climate lizards is consistent with evidence that oviparity is thermally selected against in cold areas, where low and unstable thermal conditions reduce incubation success (Shine, 2004; 2005). Viviparity, on the other hand, appears to be ecologically disadvantageous (relative to oviparity) in warm climates, as females are heavily burdened with embryos, which reduces sprinting performance, thus increasing the risk of mortality by predation (Miles *et al.*, 2000; Shine, 2005). Likewise, longer retention of embryos by viviparous females reduces the opportunities for multiple reproductive episodes, resulting in one (or less than one) litter per season (Ibargüengoytía & Cussac, 1999; Cox *et al.*, 2003; Pincheira-Donoso & Tregenza, 2011). Exceptions are found in a few oviparous *Liolaemus* that inhabit cold climates and in the viviparous *Liolaemus gravenhorstii* from warm areas. As for oviparity retention in cold climates, there is evidence that lizards might not only avoid low-temperature incubation pressures by evolving viviparity, but also that some oviparous species might actually adapt to these conditions (Shine, 2005). Oviparous species in cold climates might evolve alternative physiological and nesting strategies that provide the eggs with appropriate conditions to complete development (Shine, 1999). Also, alternative behavioural adaptations such as the evolution of communal nesting might offer optimal thermal and hydric environments for egg development (Radder & Shine, 2007). Interestingly, the (apparently) only report of communal nesting in *Liolaemus* comes from the cold climate oviparous *Liolaemus ramirezae* (c. 2800–3300 m, ALM > 47) (Espinoza & Lobo, 1996).

## The evolution and fate of viviparity

Our study reveals that viviparity in *Liolaemus* is almost entirely restricted to cold climates (Schulte *et al.*, 2000; Pincheira-Donoso *et al.*, 2008b). Therefore, viviparity is likely to have permitted the successful evolutionary radiation of these lizards into high elevations/latitudes. Remarkably, however, as environmental temperatures rise due to climate change, viviparity is set to become responsible for increases in extinction risk among cold climate *Liolaemus* species (and potentially lizards in general). This scenario is plausible because species facing environmental changes may either disperse or adapt, or experience demographic collapses that precipitate extinction if the previous two alternatives fail (Thomas *et al.*, 2004; Parmesan, 2006; Massot *et al.*, 2008). The evolution of viviparity in lizards imposes an unusual scenario where species may fail to adapt or disperse away from warming climates for two non-exclusive reasons.

First, viviparity appears to have evolved mostly in cold climates because it is thought to be ecologically too costly in warm climates where oviparous species predominate (Shine, 2005; see above). Indeed, despite the somehow paradoxical fact that longer embryo retention in viviparous species results in fitness advantages for the offspring (Shine, 1995; Shine, 2005; Ji *et al.*, 2007), viviparous species remain predominantly restricted to cold climates. Therefore, viviparity appears to be a viable strategy where selection on reproduction is so strong that the ecological costs become comparatively weaker (Shine, 2005). In other words, viviparity would be a form of emergency strategy viable primarily in cold climates where existence would otherwise be difficult. Consequently, as historically cold climates become warmer, viviparous species face at least three potential ecological crises: (1) they suffer the high reproductive costs associated with viviparity where this parity mode is not critical and hence disadvantageous (e.g. lower fecundity and reduced ability to escape predators due to egg burden); (2) they suffer unprecedented ecological competition with oviparous lizards expanding upward and poleward following the displacement of the minimum thermal threshold for oviparity tolerance (Parmesan, 2006; Massot *et al.*, 2008; Huey *et al.*, 2010; Sinervo *et al.*, 2010); and (3) they suffer energetic shortfalls associated with longer periods of retreat (hence, reduced foraging time) in cooler shelters used during hot hours of the day for behavioural thermoregulation and avoidance of mortality due to overheating (Huey *et al.*, 2010). This latter factor appears to be particularly prominent, as a recent study (Sinervo *et al.*, 2010) revealed that climate warming might increase the risk of lizard extinctions by forcing them to retreat for longer into these refuges (Huey *et al.*, 2010). Also, these forced retreats are likely to have comparatively higher detrimental consequences for viviparous species as pregnant females require high energy intakes to sustain embryo development, while foraging is more dangerous due to the pregnancy burden (Miles *et al.*, 2000; Shine, 2005).

Second, for squamates in general, viviparity is regarded as predominantly irreversible, i.e. from which oviparity is unlikely to re-evolve (Lee & Shine, 1998; Shine & Lee, 1999; Shine, 2005). Our phylogenetic evidence supports this tendency in *Liolaemus* lizards, where the unidirectional oviparity–viviparity evolutionary transition was found to be most likely among competing hypotheses (Table 2). Although some uncertainties in the employed *Liolaemus* phylogenetic hypothesis (Fig. 1) suggest that there are potential contender cases for reversions, our phylogenetic analyses reinforce the view that transitions to viviparity are significantly more likely.

For additional reasons it is likely that viviparity will be associated with an increased risk of extinction in these and other lizards facing climate change. For example, rapid climate warming may compromise embryonic development in viviparous species through elevation of the mother's active body temperatures (Beuchat, 1986; Sinervo *et al.*, 2010). Indeed, Sinervo *et al.* (2010) found that the extinction risk of viviparous Mexican lizards was twice that of their oviparous counterparts, and more strongly linked to cool montane habitats. Also, it has been suggested that species with restricted distributional ranges, particularly from high latitudes–elevations, may suffer much stronger range contractions and hence a higher risk of extinction (Parmesan, 2006; Thomas *et al.*, 2006; Pincheira-Donoso, 2011). In fact, species inhabiting these climates have already experienced high rates of extinction due to climate change

(Pounds *et al.*, 2005; Parmesan, 2006; Thomas *et al.*, 2006; Massot *et al.*, 2008).

Our analyses reveal a pattern of climatic restrictions of viviparous species in high latitudes–elevations, which are predicted to result in a higher risk of range contractions, fragmentations, and hence extinctions, compared with oviparous species. Poleward and upward displacements of maximum thermal limits for viviparity are likely to force viviparous *Liolaemus* to displace their minimum altitudinal and latitudinal ranges in the same poleward and upward directions under persistent climate warming. These range shifts are likely to progressively become contractions when retracting species encounter physical barriers that impede spatial displacements, particularly for high-elevation species approaching mountaintops (Parmesan, 2006; Thomas, 2010; Pincheira-Donoso, 2011). Since a high proportion of *Liolaemus* species are viviparous and Andean, the risk of extinction that these lizards might face under climate warming could be substantial (Pincheira-Donoso, 2011). Given that the historical distributions of *Liolaemus* have not been monitored, it is not possible to demonstrate a causative link between these distributional patters and climate warming. However, we have shown in evolutionary terms the potential for oviparous species to invade areas currently occupied by viviparous species if the biological effects of climate warming keep moving in the directions predicted by theory and supported by evidence (Parmesan, 2006).

Collectively, these ecological and genetic scenarios combined suggest that viviparity might turn out to be an evolutionary dead-end in the face of climate change. However, these predicted extinctions of viviparous species need not simply reduce *Liolaemus* diversity. We expect the genus to experience species turnovers in historically cold climates, where invasions by oviparous species (and extinction of viviparous) might drive new speciation events, resulting in new forms of high-latitude and high-elevation *Liolaemus* fauna.

## ACKNOWLEDGEMENTS

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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.** Summary of *Liolaemus* species data included in this study.

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### BIOSKETCH

**Daniel Pincheira-Donoso** is a lecturer in zoology interested in the ecological basis of adaptive evolution, macroecology and the consequences of climate change on biodiversity. He is an authority on the Liolaemid lizards of South America.

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