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VARIACIÓN DE RASGOS, TRADE-OFFS Y SU RELACIÓN CON LOS LÍMITES DE DISTRIBUCIÓN: EL CASO DE LA LAGARTIJA ANDINA *Proctoporus lacertus* (GYMNOPHTHALMIDAE) DE LOS ANDES DE PERÚ

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Dedicatoria

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RESUMEN

La variación geográfica de rasgos morfológicos, fisiológicos y comportamentales constituye una expresión fundamental de la biodiversidad, ya que refleja cómo los organismos responden a las presiones selectivas impuestas por el ambiente. En especies ampliamente distribuidas, estas diferencias fenotípicas tienden a expresarse de manera más pronunciada y pueden afectar el desempeño local en términos competitivos, energéticos, conductuales y reproductivos, influyendo en conjunto sobre la persistencia de las poblaciones a lo largo del tiempo. En ectotermos, los rasgos morfológicos como el tamaño corporal no responden a las reglas macroecológicas clásicas, sino que reflejan la interacción entre temperatura, estacionalidad climática, humedad, disponibilidad de recursos y presión de depredación. Asimismo, los rasgos térmicos pueden variar en respuesta a presiones selectivas contrastantes impuestas por la heterogeneidad térmica del ambiente. A su vez, esta variación geográfica puede dar lugar a *trade-offs*, de modo que las adaptaciones que incrementan la tolerancia ambiental pueden implicar costos en otros componentes del desempeño, como la capacidad competitiva, contribuyendo al establecimiento de los límites del rango de distribución de las especies. En este contexto, en el presente estudio evaluamos cómo *Proctoporus lacertus*, una lagartija que habita los Andes del sureste de Perú, responde a la heterogeneidad ecológica de la Cordillera de los Andes, integrando la variación del tamaño corporal, los rasgos térmicos y las interacciones competitivas con congéneres en los límites de su rango de distribución. Nuestros resultados evidencian una variación significativa en el tamaño corporal, con individuos de menor tamaño asociados a condiciones más áridas. Además, los rasgos térmicos mostraron respuestas mixtas a lo largo del gradiente altitudinal. La población de mayor elevación presentó un rango de tolerancia térmica más amplio y una mayor tolerancia al frío, mientras que la tolerancia al calor se mantuvo relativamente conservada

entre localidades. Asimismo, los rasgos térmicos voluntarios permanecieron mayormente constantes a lo largo del gradiente altitudinal, con excepción de VT_{min}, que fue menor en la población de mayor elevación. Encontramos que la reducción del tamaño corporal asociada a ambientes áridos tiene consecuencias competitivas. En experimentos de jardín común, los individuos pequeños de *P. lacertus* fueron competitivamente subordinados frente a *P*. cf. *kiziriani*, un congénere de mayor tamaño que habita la porción más elevada y húmeda del límite de distribución de *P. lacertus*. En cambio, los individuos grandes de *P. lacertus* fueron competitivamente dominantes frente a *P*. cf. *kiziriani*. En conjunto, estos resultados sugieren la existencia de un *trade-off* evolutivo, en el cual el fenotipo pequeño favorecería la persistencia en ambientes áridos, pero a costa de una menor capacidad competitiva en zonas de contacto con congéneres de mayor tamaño, limitando la expansión de su rango de distribución. Por lo tanto, los límites de distribución de *P. lacertus* no estarían determinados únicamente por restricciones climáticas directas, sino por la interacción entre adaptación local, *trade-offs* evolutivos e interacciones bióticas.

ABSTRACT

Geographic variation in morphological, physiological, and behavioral traits constitutes a fundamental expression of biodiversity, as it reflects how organisms respond to the selective pressures imposed by the environment. In widely distributed species, these phenotypic differences tend to be expressed more pronouncedly and may affect local performance in competitive, energetic, behavioral, and reproductive terms, collectively influencing population persistence over time. In ectotherms, morphological traits such as body size do not necessarily conform to classic macroecological rules, but instead reflect the interaction among temperature, climatic seasonality, humidity, resource availability, and predation pressure. Likewise, thermal traits may vary in response to contrasting selective pressures imposed by environmental thermal heterogeneity. These pressures may be particularly intense in high-elevation environments, where intense nocturnal cooling and high daytime solar radiation generate markedly contrasting thermal conditions. In turn, this geographic variation may give rise to trade-offs, whereby adaptations that increase environmental tolerance may entail costs in other components of performance, such as competitive ability, thereby contributing to the establishment of species' distributional range limits. Therefore, these trade-offs play an important role in establishing species distribution limits. In this context, the present study evaluated how *Proctoporus lacertus*, a lizard inhabiting the Andes of southeastern Peru, responds to the ecological heterogeneity of the Andean Cordillera by integrating variation in body size, thermal traits, and competitive interactions with congeners at the limits of its distributional range. Our results revealed significant variation in body size, with smaller individuals associated with more arid conditions. In addition, thermal traits showed mixed responses along the elevational gradient. The high-elevation population exhibited a broader thermal tolerance range and greater cold tolerance, whereas heat tolerance

remained relatively conserved among localities. Likewise, voluntary thermal traits remained largely constant along the elevational gradient, with the exception of VT_{min}, which was lower in the high-elevation population. We also found that the reduction in body size associated with arid environments has competitive consequences. In common garden experiments, small individuals of *P. lacertus* were competitively subordinate to *P.* cf. *kiziriani*, a larger congener that inhabits the higher and more humid portion of the distributional limit of *P. lacertus*. Conversely, large individuals of *P. lacertus* were competitively dominant over *P*. cf. *kiziriani*. Overall, these results suggest the existence of an evolutionary trade-off, in which the small phenotype may favor persistence in arid environments, but at the cost of reduced competitive ability in contact zones with larger congeners, thereby limiting the expansion of its distributional range. Consequently, the distribution limits of *P. lacertus* may not be determined solely by direct climatic constraints, but rather by the interaction among local adaptation, evolutionary trade-offs, and biotic interactions.

INTRODUCCIÓN GENERAL

La variación geográfica de rasgos (morfológicos, fisiológicos y comportamentales) es una propiedad ubicua de los sistemas naturales y una de las expresiones más informativas de la biodiversidad (Gould & Johnston, 1972). Estas diferencias reflejan la interacción entre procesos ecológicos y evolutivos que operan a múltiples niveles de organización y constituyen evidencias de cómo los organismos responden a presiones selectivas de gradientes ecológicos y paisajes heterogéneos, y de cómo la historia demográfica y biogeográfica puede dejar rastros persistentes en la estructura del fenotipo a través del tiempo y espacio (Endler, 1977; Gould & Johnston, 1972; Zamudio et al., 2016).

Las especies ampliamente distribuidas suelen diferir de manera sistemática en rasgos morfológicos (p. ej., tamaño corporal, coloración), fisiológicos (p. ej., tolerancias térmicas e hídricas, metabolismo), de historia de vida (p. ej., fecundidad, longevidad) y comportamentales (p. ej., migración, territorialidad) (Endler, 1977; Garland & Adolph, 1991). Este patrón de heterogeneidad fenotípica es una respuesta a las presiones selectivas impuestas por el ambiente, las cuales se traducen en diferencias en el desempeño, modulando la captación de energía, el balance hídrico, la locomoción, la actividad y la inversión reproductiva (Arnold, 1983; Farine et al., 2015). En conjunto, estos componentes determinan el *fitness* y por tanto explican por qué ciertos fenotipos se mantienen en diferentes contextos ambientales (Arnold, 1983; Farine et al., 2015). De esta manera, la variación geográfica de rasgos resume el vínculo mecanicista entre ambiente, desempeño y fitness, que establece la base para la persistencia y estabilidad de las especies y poblaciones a lo largo del tiempo.

Las clinas y gradientes son un marco clásico para entender patrones espaciales de variación de rasgos, donde cambios continuos en el ambiente generan cambios continuos en el fenotipo. En este contexto, se han propuesto varias hipótesis macroecológicas para

explicar la variación geográfica de rasgos morfológicos y fisiológicos. Entre las hipótesis macroecológicas más influyentes se encuentra la regla de Bergmann, originalmente formulada para endotermos, según la cual climas fríos favorecerían tamaños corporales mayores debido a su menor relación superficie/volumen, lo que facilitaría la conservación del calor corporal (Watt et al., 2010).

En ectotermos, la variación del tamaño corporal rara vez sigue una regla única, y suele emerger de la interacción entre temperatura, disponibilidad hídrica, estacionalidad y productividad ambiental. La temperatura puede influir en el tamaño corporal a través de la regla temperatura–tamaño, según la cual los ambientes cálidos incrementan la tasa metabólica y aceleran el desarrollo, reduciendo así el tiempo disponible para el crecimiento y favoreciendo la formación de adultos de menor tamaño. En contraste, las temperaturas frías pueden prolongar el desarrollo y permitir un mayor crecimiento corporal antes de la madurez. Sin embargo, ambos escenarios pueden implicar costos en términos de *fitness* por mayor exposición a riesgos durante el desarrollo o por mayor necesidad de forrajeo bajo crecimiento rápido (Atkinson, 1994; Atkinson & Sibly, 1997; Gotthard, 2001). La estacionalidad, a su vez, impone presiones contrapuestas: estaciones de crecimiento cortas favorecen un desarrollo rápido y tamaños menores (Atkinson & Sibly, 1997; Gotthard, 2001), pero periodos predecibles de escasez pueden seleccionar tamaños mayores por su capacidad de acumular reservas (Adolph & Porter, 1996). La disponibilidad hídrica también puede influir sobre el tamaño corporal, ya que en ambientes áridos ciertos fenotipos de mayor tamaño podrían reducir la pérdida relativa de agua (Nevo, 1973). Además, en ovíparos, la limitación hídrica durante la incubación puede restringir la absorción de agua por el embrión, disminuir el tamaño del huevo y prolongar el desarrollo, reduciendo el éxito de eclosión y produciendo crías más pequeñas y de peor condición, con posibles efectos negativos sobre su desempeño (Bell et al., 2025;

Du, 2004). Finalmente, la productividad y la depredación determinan la energía disponible y el riesgo asociado al forrajeo, favoreciendo tamaños menores en ambientes pobres o con alta depredación y tamaños mayores en ambientes productivos, aunque potencialmente bajo una mayor presión de depredación (Blanckenhorn, 2000; Taylor & Cox, 2019).

Además de la variación morfológica, los gradientes altitudinales y latitudinales también moldean los rasgos fisiológicos porque responden a cambios de los componentes del clima, generando gradientes de selección que se expresan como variación geográfica de rasgos térmicos. Sin embargo, aunque estos gradientes pueden favorecer divergencia fisiológica, su magnitud puede verse atenuada por la termorregulación conductual, que amortigua la exposición a extremos térmicos. A lo largo de gradientes latitudinales, los ectotermos suelen exhibir un patrón macrofisiológico consistente: la amplitud de tolerancia térmica aumenta hacia latitudes altas, principalmente porque los límites de tolerancia al frío (p. ej., CT_{min}) se desplazan a temperaturas más bajas conforme se avanza hacia los polos, mientras que los límites al calor (CT_{max}) tienden a cambiar poco en muchos taxones terrestres (Sunday et al., 2019). En montañas tropicales, la baja estacionalidad se ha asociado con tolerancias térmicas más estrechas y barreras fisiológicas más fuertes (Janzen, 1967; Sheldon et al., 2018). Sin embargo, en elevaciones altas la radiación diurna intensa y el enfriamiento nocturno pueden generar amplitudes térmicas diarias marcadas, incluso con temperaturas nocturnas bajo 0 °C, imponiendo selección tanto por frío como por calor y favoreciendo mayores amplitudes térmicas en altura (hipótesis de variabilidad climática de Janzen) (Muñoz & Bodensteiner, 2019; Reider et al., 2021). En conjunto, estos patrones sugieren un desacople entre límites inferiores y superiores, donde el frío impone restricciones más recurrentes sobre la actividad y la supervivencia, favoreciendo una mayor labilidad de los rasgos térmicos

inferiores, mientras que rasgos térmicos superiores permanecen relativamente conservados, conocido como la hipótesis de Brett (Brett, 1956; Gaston et al., 2009). No obstante, la divergencia fisiológica puede estar limitada porque la termorregulación conductual amortigua la exposición a condiciones extremas y reduce la intensidad de la selección natural sobre la fisiología térmica (efecto Bogert), manteniendo los rasgos voluntarios relativamente similares a través de gradientes climáticos como la temperatura de selección (T_{sel}), los límites conductuales (VT_{min}, VT_{max}), y CT_{max} (Bogert, 1949; Huey et al., 2003; Muñoz, 2022).

Sin embargo, la variación geográfica de rasgos a lo largo de gradientes climáticos puede tener consecuencias directas sobre el *fitness* y generar compromisos evolutivos (*trade-offs*), los cuales surgen cuando la mejora de un rasgo ocurre a expensas de otro por restricciones de tiempo, energía o función, de modo que ningún fenotipo puede maximizar simultáneamente todos los componentes del *fitness* (Stearns, 1989). En consecuencia, estos compromisos pueden contribuir al establecimiento de los límites del rango de distribución al restringir la persistencia o la expansión en ambientes contrastantes (Willi & Van Buskirk, 2022). Por ejemplo, reducciones en el tamaño corporal pueden traducirse en menor fecundidad porque el tamaño de la hembra limita el espacio y la energía disponibles para producir o portar descendencia, generando una asociación positiva entre tamaño corporal y fecundidad (Blanckenhorn, 2000; Monroe et al., 2015). De manera similar, un aumento de la tolerancia a estresores abióticos como el calor o el frío puede implicar costos en otros componentes del fitness, ya que una mayor inversión en tolerancia y mantenimiento puede reducir el desempeño en crecimiento o reproducción (Willi & Van Buskirk, 2022). Además, existe evidencia de que, en algunos contextos, una mayor tolerancia al estrés puede estar asociada con una menor capacidad competitiva, donde especies o genotipos más tolerantes pueden rendir peor bajo

competencia frente a residentes, haciendo plausible que la colonización de nuevos ambientes ocurra a expensas del desempeño competitivo (Qi et al., 2018). Finalmente, las tasas de crecimiento elevadas suelen estar condicionadas por la energía disponible y pueden acarrear costos persistentes como menor rendimiento posterior, reforzando compromisos entre crecimiento, reproducción y supervivencia (Perez & Munch, 2015). En conjunto, la variación de rasgos y sus costos asociados ayudan a explicar por qué los bordes de rango pueden emerger allí donde el estrés ambiental y las interacciones bióticas reducen la persistencia local.

En este contexto, *Proctoporus lacertus* es una lagartija semifosorial de la vertiente oriental de los Andes del sureste del Perú (Apurímac, Ayacucho, Cusco), con un rango altitudinal amplio (2500–4300 m) y marcada variación geográfica en tamaño corporal. Las poblaciones altoandinas de puna húmeda y bosque montano son más grandes y robustas, mientras que las poblaciones de valles interandinos son más pequeñas y esbeltas (Doan & Castoe, 2003; Goicoechea et al., 2013; Mamani, 2020). La variación ha sido tan pronunciada que motivó confusiones taxonómicas, incluyendo el reconocimiento de la forma asociada a valles interandinos como *P. sucullucu* (Doan & Castoe, 2003) que posteriormente, basada en evidencia molecular, fue sugerida como sinónimo de *P. lacertus* (Mamani, 2020). Además, existen estudios previos que describen termoconformismo, nidos comunales, filopatría y una CT_{max} elevada (39.4 °C), pero basados en poblaciones orientales de valle interandino (~2800 m), por lo que su generalización a todo su rango es incierta dada la variación intraespecífica esperable (Gould & Johnston, 1972). A escala ecológica, *P. lacertus* habita múltiples ecosistemas (bosque montano, matorrales áridos, valle interandino, puna seca y húmeda) y en sus límites inferiores coexiste con varios congéneres como: *P. optimus*, *P. unsaacae*, *P. machupicchu* y *P. chasqui*; mientras que en el límite superior no interactúa con otros

gimnoftalmidos. Sin embargo, al noroeste de su distribución en el Valle Sagrado de los Incas (Cusco), una población de valle interandino de cuerpo pequeño es reemplazada desde ~3560 m por un congénere no descrito (*Proctoporus* gr. *kiziriani*) de mayor tamaño (Doan & Castoe, 2003). En conjunto, su baja capacidad de dispersión, el amplio rango altitudinal que ocupa, la marcada variación geográfica del tamaño corporal y el reemplazo espacial con congéneres en algunos bordes de distribución hacen de *P. lacertus* un sistema adecuado para evaluar las siguientes preguntas: (i) ¿Qué factores ecológicos explican mejor la variación del tamaño corporal?; (ii) ¿Cómo varían los rasgos térmicos a través de un gradiente altitudinal?; (iii) ¿Los *trade-offs* asociados a los fenotipos de ambientes áridos limitan la expansión del rango de distribución?

Predicciones

Predicción 1: la reducción del tamaño corporal en ambientes áridos como los valles interandinos, se asociará principalmente con restricciones hídricas.

Predicción 2: La temperatura crítica mínima (CT_{min}) variará más que la máxima (CT_{max}) a lo largo del gradiente altitudinal, de modo que el rango de tolerancia térmica (R_{tol}) será mayor en los ambientes más elevados. En contraste, las temperaturas voluntarias (T_{sel}, VT_{min}, VT_{max}, y R_{vol}) se mantendrán relativamente conservadas a lo largo del gradiente altitudinal.

Predicción 3: La reducción del tamaño corporal asociada a la aridez disminuirá la capacidad competitiva de *P. lacertus* frente a *P.* gr. *kiziriani*, de modo que en las zonas de contacto *P. lacertus* tenderá a mostrar un menor desempeño competitivo, lo que limitará la colonización de ambientes más elevados y restringirá su rango de distribución.

Objetivo general

Evaluar como la variación del tamaño corporal, los rasgos térmicos y los *trade-offs* asociados a *Proctoporus lacertus* se relacionan con el establecimiento de los límites de su rango de distribución.

Objetivo especifico

- Evaluar los determinantes climáticos y ecológicos de la variación del tamaño corporal de *Proctoporus lacertus*.
- Evaluar la variación de los rasgos térmicos de *P. lacertus* a través de un gradiente altitudinal.
- Evaluar las consecuencias competitivas de la reducción del tamaño corporal de *P. lacertus* frente *P.* gr. *kiziriani*.

Este capítulo ha sido sometido para evaluación en la revista *Journal of Zoology*

1. CAPÍTULO I: ARIDITY REDUCES BODY SIZE OF A SEMI-FOSSORIAL LIZARD FROM THE PERUVIAN ANDES

1.1 Abstract

Body size is a key trait associated with physiological, ecological and evolutionary processes that influence organismal performance and survival. In ectotherms, its variation is shaped by gradients of temperature, water availability, climatic variability and productivity, although these factors do not act universally. In this study, we evaluated body size variation and its associated proximate ecological factors in *Proctoporus lacertus*, a semifossorial lizard distributed in the Andes of southeastern Peru. We measured 16 morphometric variables in 239 individuals and used the first principal component from a principal component analysis as an integrated measure of body size. We then evaluated body size variation among vegetation units using ANOVA and analyzed the influence of ecological variables using LLMs. Body size varied consistently among vegetation types, with larger individuals occurring in humid environments than in arid environments. Similarly, sexual dimorphism was environment-dependent, as males were larger than females only in humid environments. Climatic models showed that climatic moisture index (CMI) and thermal seasonality (BIO4) significantly explained body size variation; however, vegetation cover explained the largest proportion of this variation. These results suggest that body size in *P. lacertus* does not respond to a single environmental predictor, but rather to a combination of thermal, hydric and structural gradients, highlighting the importance of Andean environmental heterogeneity in generating phenotypic variation.

Keywords: Andean reptiles; aridity; body size; climatic moisture; environmental gradients; sexual dimorphism; vegetation structure; *Proctoporus lacertus*.

1.2. INTRODUCTION

Body size is one of the most important traits in organismal biology because of its relationship with physiological, ecological and evolutionary processes that are essential for species survival and persistence (Blanckenhorn, 2000; Darwin, 1871; Kingsolver & Huey, 2008). In many animals, larger body size often confers advantages such as greater capacity for territorial defense, access to mates, foraging efficiency and resistance to periods of resource scarcity (Blanckenhorn, 2000; Darwin, 1871; Kingsolver & Huey, 2008). However, it also entails costs, including higher energetic requirements, reduced agility, greater exposure to predators and prolonged development times (Blanckenhorn, 2000; Combes et al., 2013; Gotthard, 2001).

Although geographic variation in body size has been interpreted under general macroecological rules, such as Bergmann's rule (Bergmann, 1948), its applicability to ectotherms is less consistent (Ashton & Feldman, 2003; Mousseau, 1997; Shelomi, 2012). In these organisms, body size often depends on the interaction among ecological variables such as temperature, water availability, seasonality and productivity (Atkinson, 1994; Atkinson & Sibly, 1997; Blanckenhorn, 2000; Gotthard, 2001). This dependence suggests that body size variation does not respond to a single ecological factor, but rather to multiple factors that may impose contrasting selective pressures. For example, high temperatures can accelerate metabolism and development, reducing the time available for growth and favouring smaller adult body sizes (Atkinson, 1994; Atkinson & Sibly, 1997). In contrast, low temperatures can slow these processes, prolong the growth period and favour larger adult body sizes (Atkinson & Sibly, 1997; Blanckenhorn, 2000). However, both prolonged development and accelerated growth may entail costs. The former increases the time individuals remain exposed to predators, parasites and starvation before reproduction, whereas the latter can increase energetic demands and the need for foraging,

thereby increasing exposure to predators and the risk of energetic deficit when resources are limited (Blanckenhorn, 2000; Gotthard, 2001; Gotthard et al., 1994).

Seasonality can also modulate body size variation by altering the duration of periods favourable for growth, activity and reproduction. In highly seasonal environments, growth windows are often brief, limiting the time available to reach reproductive maturity. Under these conditions, selection may favour individuals that complete development rapidly, even if this results in smaller adults (Atkinson & Sibly, 1997; Gotthard, 2001). In contrast, in environments with longer favourable seasons or more thermally stable conditions, organisms may have more time to invest in growth and attain larger sizes at maturity (Atkinson, 1994; Atkinson & Sibly, 1997; Gotthard, 2001). However, seasonality affects not only growth rates, but also energy-storage strategies. When periods of resource scarcity are predictable and prolonged, larger body size may be beneficial by allowing individuals to accumulate sufficient energy reserves to withstand starvation during unfavourable seasons (Adolph & Warren, 1996; Gotthard, 2001). Therefore, seasonality may impose contrasting selective pressures: whereas a short growing season may favour early maturation and reduced body size, the need to tolerate prolonged periods of low resource availability may favour larger body sizes.

Water availability is another climatic variable closely associated with body size variation. In arid environments, larger body sizes may be advantageous because it reduces the relative surface area exposed to the environment, thereby decreasing water loss through evapotranspiration and allowing greater accumulation of water reserves (Nevo, 1973). This mechanism has been proposed mainly for amphibians, whose activity often occurs at night and near water bodies, where evaporative conditions are less intense (Johnson et al., 2023). However, in diurnal organisms, a large body may also impose costs in dry and warm environments, as it can increase the risk of overheating and reduce the efficiency

of heat dissipation, thereby compromising water balance and survival. Some groups possess traits that mitigate these effects; for example, in beetles, a thicker cuticle may reduce evapotranspiration (Ospina-Rozo et al., 2024), whereas in reptiles, the accumulation of subcutaneous adipose tissue may reduce water loss through evapotranspiration (Roberts & Lillywhite, 1980). In addition, water availability can influence body size from embryonic development onwards. Low water availability during this period may reduce water uptake by the egg and restrict embryonic growth, compromising both viability and body size at hatching (Du, 2004). Consequently, lower hatching success and smaller hatchlings may occur, with negative effects on subsequent performance (Bell et al., 2025; Du, 2004; Wayne et al., 2025).

Resource availability and predation pressure can also influence body size variation. In environments with low resource availability, large organisms may experience undernutrition during critical stages of development, reducing growth rates and resulting in adults with smaller body size and lower performance (Atkinson & Sibly, 1997; Blanckenhorn, 2000). Under these conditions, chronically resource-poor environments with high predation pressure tend to favour early maturation as a strategy to maximize survival (Blanckenhorn, 2000; Pérez-Tris et al., 2004). In contrast, in environments with greater resource availability, individuals may sustain higher growth rates, accumulate larger energy reserves and attain larger body sizes, with potential benefits for reproductive success (Muraro et al., 2022; Taylor & Cox, 2018). Indeed, ecosystems with high primary productivity often support higher predator densities, which may impose additional selective pressures on body size and restrict the time available for foraging (Altwegg, 2002; Dmitriew & Rowe, 2005). Moreover, undernourished organisms often take greater risks while foraging, increasing their vulnerability to predation due to poorer body condition and reduced escape capacity (Moran et al., 2021).

The tropical Andes provide a particularly suitable setting for evaluating these mechanisms, because they encompass steep thermal, hydric and vegetation gradients over short geographic distances. In this context, *Proctoporus lacertus* (Stejneger, 1913) represents a relevant study system. This semifossorial lizard inhabits the eastern slopes of the Andes in southeastern Peru, from approximately 13°04′S, where the Andes begins to widen, to 14°20′S, and between 73°20′W and 72°17′W (Fig. 1), across an elevational range of 2500–4300 m a.s.l. (Doan & Castoe, 2003; Goicoechea et al., 2012; Mamani, 2020). Throughout its distribution, this species occupies a wide variety of habitats: montane forests and humid Andean grasslands in the north; arid Andean grasslands and inter-Andean shrublands in the south and west; and humid Andean grasslands, montane forests and inter-Andean shrublands in the east (Fig. 1). Populations show marked morphological variation in body size and coloration: individuals from montane forests and humid Andean grasslands are larger and more robust, with black ventral coloration; those from inter-Andean shrublands are smaller and slenderer, with cream or reddish ventral coloration; and those from arid Andean grasslands have the smallest body sizes and cream-coloured venters with dark blotches (Fig. S1). This conspicuous variation has generated taxonomic confusion (Doan & Castoe, 2003; Goicoechea et al., 2012), leading populations from the eastern boundary of the distribution, in Ollantaytambo, Cusco, to be described as *Proctoporus sucullucu* Doan & Castoe, 2003. However, molecular evidence later showed that this taxon is a synonym of *P. lacertus* (Mamani, 2020). In addition, this species is characterized by the use of communal nests with flexible-shelled eggs and philopatric behaviour, and it tolerates relatively high temperatures, with a reported critical thermal maximum of approximately 39.4 °C, suggesting low susceptibility to the effects of climate change, at least in adults (Doan et al., 2022; Doan et al., 2021).

Despite this conspicuous variation, morphological variation in *P. lacertus* remains poorly quantified, and it is still unclear whether sexual dimorphism differs among environments or which ecological factors explain spatial patterns of body size. In this study, We (1) evaluated body size variation and sexual dimorphism across environmental gradients; (2) identified the ecological variables that best explain body size variation; and (3) examined whether vegetation cover contributes to explaining spatial variation in body size.

1.3. MATERIALS AND METHODS

Study area

The material analyzed comes from several localities in southeastern Peru, specifically from the departments of Apurímac, Ayacucho and Cusco (Fig. 1). This region is characterized by marked geographic and ecological heterogeneity, comprising a mosaic of landscapes that includes inter-Andean valleys and arid Andean grasslands on the southwestern slopes, humid montane forests to the north, and montane forest systems with semi-arid valleys in the northeast (Fig. 1).

Sampling

We examined and identified 239 adult specimens of *Proctoporus lacertus* deposited in the scientific collections of the Museo de Biodiversidad del Perú (MUBI) and the Asociación Pro-Fauna Ayacucho (PFAUNA). Of these, 129 were females and 110 were males. Regarding their origin, eight males and 11 females came from arid Andean grassland, 40 males and 44 females from inter-Andean shrublands, 22 males and 24 females from humid Andean grassland, and 40 males and 50 females from montane forest (Table S1, Fig. S2).

For all specimen, we recorded 16 morphometric variables using a Mitutoyo 500-762-20 digital caliper with a precision of 0.02 mm. These variables were: snout–vent length, head width, head height, head length, measured as snout–tympanum length, snout–forelimb length, chest width, thorax length, upper arm length, forearm length, hand length, anal plate width, pelvic width, pelvic height, thigh length, shank length, and foot length. Tail length was not included because it may be biased by autotomy and regeneration.

Environmental variables

Using the geographic coordinates of each individual, we obtained climatic information from the CHELSA database (Climatologies at High Resolution for the Earth's Land Surface Areas) (Karger et al., 2017). Based on theoretical and ecological background, we selected four predictor of body sizes: annual mean temperature (BIO1), temperature seasonality (BIO4), mean climatic moisture index (CMI) as a proxy of water availability, and mean normalized difference vegetation index (NDVI) as proxy of productivity. NDVI values were obtained from the MODIS MOD13Q1 product, available through the Google Earth Engine platform. This product provides NDVI estimates every 16 days for the period from 2000 to 2024 (Kamel et al., 2015).

To characterize the vegetation cover associated with the study species, we used the classification proposed by the Peruvian Ministry of the Environment (MINAM, 2015), with some modifications. Agricultural areas were incorporated into the corresponding vegetation-cover category; relict conifer forests and non-Amazonian forest areas were grouped under the category of altimontane mountain forest; and Andean grassland was subdivided into two types: humid Andean grassland, located immediately above montane forests, and arid Andean grassland, characterized by its occurrence above arid inter-Andean shrublands (Figs 1, S2).

Statistical Analyses

We performed a principal component analysis (PCA) on the 16 morphometric variables using the *stats* package in R v.4.5.1 (R Core Team, 2025). The first principal component (PC1), which retained 77% of the variation contained in the 16 variables, was used as a measure of body size in subsequent analyses (Fig. S3; Table S1).

To evaluate the effects of sex, vegetation type and their interaction on body size, we fitted a two-way ANOVA using heteroscedasticity-consistent variance–covariance estimators (HC3), using the *sandwich* package (Zeileis, 2004). This approach was used to obtain robust standard errors in response to the heteroscedasticity detected in the data. Post-hoc comparisons were conducted using estimated marginal means with Tukey adjustment in the *emmeans* package (Lenth & Piaskowski, 2025). Normality and homoscedasticity were evaluated using Shapiro–Wilk and Levene's tests, implemented in the *stats* and *car* packages (Fox & Weisberg, 2018), respectively. Normality was assessed separately for sex and vegetation type and was met in all cases ($p > 0.05$). However, Levene's test detected heteroscedasticity ($F_{7, 231} = 4.59$, $p < 0.001$).

To examine the environmental factors associated with body-size variation in *Proctoporus lacertus*, we fitted two linear mixed-effects models (LMMs). In both models, PC1 was used as the response variable, while sex, annual mean temperature (BIO1), temperature seasonality (BIO4), climatic moisture index (CMI), and mean NDVI were included as fixed effects. Climatic pixels were incorporated as random intercepts to account for the non-independence of individuals sampled within the same pixel and for unequal sample sizes among pixels. The first model (M1) included only climatic and productivity predictors, whereas the second model (M2) additionally included vegetation category as a fixed effect. Vegetation was incorporated because habitat and microhabitat structure may proximally integrate the effects of regional climate, resource availability, and refuge

conditions. All continuous predictors were z-standardized to a mean of 0 and a standard deviation of 1, facilitating model interpretation and direct comparison of their effect sizes. Models were fitted using the *nlme* package (Pinheiro et al., 2025) and estimated by restricted maximum likelihood (REML), which reduces the small-sample bias in the estimation of variance components relative to conventional maximum likelihood (Pinheiro & Bates, 2000).

Multicollinearity was assessed using the variance inflation factor (VIF). All predictors showed VIF low values, indicating low collinearity among the explanatory variables included in the models. Finally, spatial autocorrelation in model residuals was evaluated using Moran's I with the *spdep* package. This analysis did not detected positive spatial autocorrelation in the residuals of either models (Moran's I = 1.485, $p = 0.068$ for M1; Moran's I = 1.041, $p = 0.149$ for M2).

All statistical analyses were performed in R, and figures were produced using the *ggplot2* package (Wickham, 2016). Significance for all test statistics was set at $p < 0.05$.

1.4. RESULTS

Body size variation by sex and vegetation

Vegetation type ($F_{3,231} = 122.38$, $p < 0.001$) and sex ($F_{1,231} = 13.67$, $p < 0.001$), as well as their interaction ($F_{3,231} = 4.86$, $p < 0.003$) influenced body size of lizards (two-way ANOVA with robust estimators).

Post-hoc comparisons between sexes within each vegetation type, using Tukey adjustment, showed that body size differed between sexes in montane forest ($\Delta = -2.152 \pm 0.640$, $p < 0.001$) and humid Andean grasslands ($\Delta = -2.578 \pm 0.653$, $p < 0.001$), where

males had larger body size than females. In contrast, no differences between sexes were found in arid Andean grasslands ($\Delta = 0.368 \pm 0.838$, $p = 0.661$) or inter-Andean shurblands ($\Delta = -0.405 \pm 0.352$, $p = 0.251$) (Fig. 2).

Post-hoc comparisons between vegetation types within each sex, using Tukey adjustment, showed differences in body sizes (Table 1). In females, individuals from montane forest and humid Andean grassland had larger body sizes than those from arid Andean grassland and inter-Andean shrublands ($p < 0.015$). In males, differences were even more pronounced, with larger body sizes in humid environments than in arid environments ($p < 0.001$). No differences were detected between humid montane forest and humid Andean grassland, or between arid Andean grassland and inter-Andean shrublands (Table 1).

Environmental effects on sex-adjusted body size

For model M1, which included sex and environmental predictors, sex, CMI, and BIO4 were significantly associated with body size. Males were larger than females, while the positive coefficients for CMI and BIO4 indicated that individuals tended to attain larger body sizes in localities with greater water availability and higher thermal seasonality (Table 2; Fig. 3). In contrast, BIO1 and NDVI were not significantly associated with body size (Fig. 4).

For model M2, which additionally included vegetation type as a factor, the effect of vegetation was highly significant, whereas the climatic variables were not significant (Table 2, Fig. 4). Moreover, individuals from montane forest and humid Andean grassland showed larger body sizes than those from the reference vegetation type, arid Andean grassland, whereas inter-Andean shrublands did not differ from the reference category (Table 2).

1.5. DISCUSSION

Our results show that body size in *Proctoporus lacertus* varied among vegetation formations, with larger individuals occurring in montane forests and humid Andean grasslands, and smaller individuals occurring in arid Andean grasslands and inter-Andean shrublands (Fig. 2). In addition, sexual size dimorphism was expressed only in the more humid vegetation formations, where males were larger than females, whereas this difference disappeared in arid environments.

Humid vegetation formations occur mainly in the northern portion of the species' distribution, where habitats are characterized by low evapotranspiration rates, high environmental humidity associated with persistent cloud cover and higher primary productivity (Bruijnzeel, 2001; Oliveras et al., 2014). These conditions may promote greater resource availability and stability, thereby favouring the development of larger body sizes. In contrast, the arid Andean grasslands of the south and the inter-Andean shrublands are characterized by marked thermal fluctuations, higher evapotranspiration rates, low humidity and high climatic seasonality (Killeen et al., 2007; Milla et al., 2025; Perry et al., 2014). In these environments, high daytime temperatures could increase metabolic rates while simultaneously restricting temporal activity periods due to the risk of overheating. Together with lower food-resource availability, this scenario could limit growth and favour smaller body sizes (Atkinson, 1994; Atkinson & Sibly, 1997; Blanckenhorn, 2000).

The fact that sexual size dimorphism was expressed only in montane forests and humid Andean grasslands suggests that the expression of this difference between sexes is strongly conditioned by the environment. In productive environments, greater resource availability and stability may allow individuals to attain better body condition and enable males to allocate more energy to growth and traits associated with sexual competition

(Bonduriansky, 2007). In *Podarcis siculus*, sexual size dimorphism was found to increase in populations with higher body condition and greater primary productivity (Muraro et al., 2022). In this context, although our study did not directly evaluate the mechanisms underlying sexual size dimorphism, our results suggest that humid environments, such as montane forests and humid Andean grasslands, may provide greater resource availability, allowing the expression of sexual size dimorphism in *Proctoporus lacertus*, whereas arid environments, such as inter-Andean shrublands and arid Andean grasslands, with ecologically stressful conditions, may limit its expression.

Regarding the ecological factors associated with body size variation, in the first model (M1), the climatic moisture index and thermal variation consistently explained body size variation in *P. lacertus*, with positive and significant effects (Figs 3, 4). Thus, larger individuals occurred mainly in wetter and more climatically variable environments, whereas annual mean temperature and the normalized difference vegetation index did not explain this variation. This result is consistent with findings for *Zootoca vivipara louislantzi*, in which body mass variation is also positively related to water availability (Chaubaud et al., 2022). However, it contrasts with patterns reported for other lizard lineages, such as *Sceloporus*, where body size has been positively associated with maximum temperature and aridity (Oufiero et al., 2011), and *Anolis carolinensis*, where larger males occur at sites with higher mean temperatures (Toyama et al., 2023). In contrast, body size in North American salamanders and in the Atacama Desert toad, *Rhinella atacamensis*, varies positively and consistently with humidity (Durán et al., 2021; Fleming & Sheldon, 2025). In *P. lacertus*, its semifossorial habits and the presence of flexible-shelled eggs may make humidity a particularly relevant factor. The refuges and nesting sites used by this species probably play an important role in shaping body size. Humid and relatively cool microhabitats that characterize the environments used by

P. lacertus could prolong embryonic development and promote slower growth rates, potentially resulting in larger adult body sizes (Bell et al., 2025; Du, 2004; Wayne et al., 2025). However, because annual mean temperature was not significant, thermal mechanisms probably operate at the microenvironmental scale rather than at the macroclimatic scale. These differences suggest that the relationship between climate and body size strongly depends on the ecological, life history and evolutionary history of each lineage.

Thermal seasonality was the second most important predictor explaining body size variation of *P. lacertus*, with larger individuals occurring in environments with greater thermal variability. This pattern is consistent with findings for females of *Zootoca vivipara*, a lizard widely distributed across Europe and Asia, in which body size is also associated with climatic seasonality, although responses differ among lineages (Roitberg et al., 2020). Similarly, in Darwin's frog, *Rhinoderma darwinii*, the positive association between body size and climatic seasonality has been interpreted under the starvation-resistance hypothesis, whereby larger body size may improve tolerance to prolonged periods of inactivity or hibernation by allowing greater accumulation of energy reserves (Valenzuela-Sánchez et al., 2015). However, this mechanism seems unlikely in *P. lacertus*, given that it is a tropical Andean species for which there is no evidence of prolonged inactivity comparable to hibernation or aestivation. On the contrary, year-round activity has been reported, at least in inter-Andean shrublands populations (Doan et al., 2021). Therefore, the positive relationship between body size and thermal seasonality in *P. lacertus* is unlike to be exclusively attributable to starvation resistance. Nevertheless, this mechanism cannot be completely ruled out, particularly considering that this region experiences thermal and hydric stress during winter (June–August) (Imfeld et al., 2021), which could temporarily reduce resource availability.

For the second model (M2), vegetation cover was the strongest predictor of body size variation in *P. lacertus*, exceeding the importance of the other environmental predictors, including CMI and BIO4 (Fig. 4). Vegetation cover, in addition to influencing local temperature and humidity, is associated with the availability of trophic resources and refuges, thereby affecting the thermal and energetic performance of individuals. In humid environments, vegetation structure could generate microclimates with lower thermal variation and greater moisture retention, conditions consistent with slower and more prolonged growth resulting in larger body sizes (Blanckenhorn, 2000). In addition, in productive ecosystems such as montane forests, the greater abundance of invertebrates may increase the energy available for growth and reproduction, favouring individuals with better body condition (Altwegg, 2002; Muraro et al., 2022). In contrast, arid environments, characterized by extreme thermal conditions, low humidity, low productivity and high seasonality, impose strong physiological constraints on growth (Adolph & Warren, 1996). Under these conditions, water loss during embryonic development and prolonged exposure to high temperatures may increase maintenance costs and reduce the effective time available for feeding and development. Together with low vegetation productivity and resource scarcity, these factors may generate selective pressures favouring smaller body sizes and early maturation (Blanckenhorn, 2000; Moran et al., 2021). This scenario may also be reinforced by the presence of predators such as *Tachymenis peruviana* in inter-Andean shrublands, which could exert additional selective pressure on body size by restricting activity and foraging periods (Altwegg, 2002; Taylor & Cox, 2018). Although our study did not explicitly evaluate this factor, predation pressure may act as an additional and relevant modulator of selection on body size of *P. lacertus*. Likewise, the lack of significant NDVI effects should be interpreted with caution. Persistent cloud cover in humid montane forests and humid Andean grasslands

may have reduced the accuracy of satellite-derived estimates, limiting the ability of NDVI to adequately capture vegetation cover and actual productivity in these environments.

Together, our findings indicate that body size variation in *P. lacertus* is associated with water availability, thermal seasonality and vegetation cover, showing that multiple environmental and ecological factors interact to shape this trait. The fact that vegetation cover explained the largest proportion of variation in M2 suggests that phenotypic responses in this species are more closely linked to local and microclimatic conditions than to broad scale climatic averages. Overall, these results provide empirical evidence that Andean ecological gradients may impose contrasting selective pressures on body size in Andean reptiles, highlighting the importance of environmental heterogeneity in generating phenotypic variation across mountain systems.

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Figure 1.1 Geographic distribution of *Proctoporus lacertus*. Coloured points indicate the ecological origin of the sampled individuals: yellow for humid Andean grassland (HAG), light blue for inter-Andean shrubland (IAS), red for arid Andean grassland (AAG) and light green for humid montane forest (HMF).

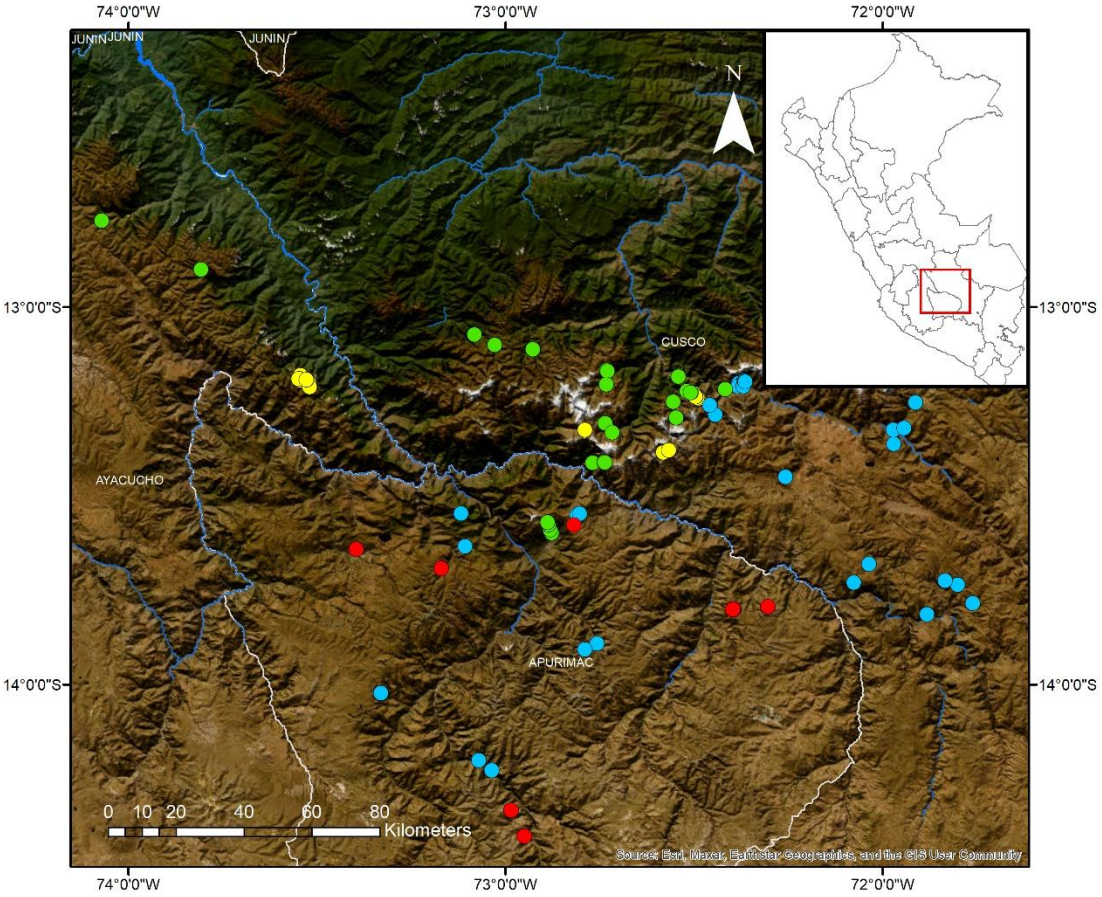


Figure 1.2 Body size variation of *Proctoporus lacertus* by vegetation cover and sex. Asterisks indicate statistically significant differences between sexes within the same vegetation type. HAG, humid Andean grassland; IAS, inter-Andean shrubland; AAG, arid Andean grassland; HMF, humid montane forest.

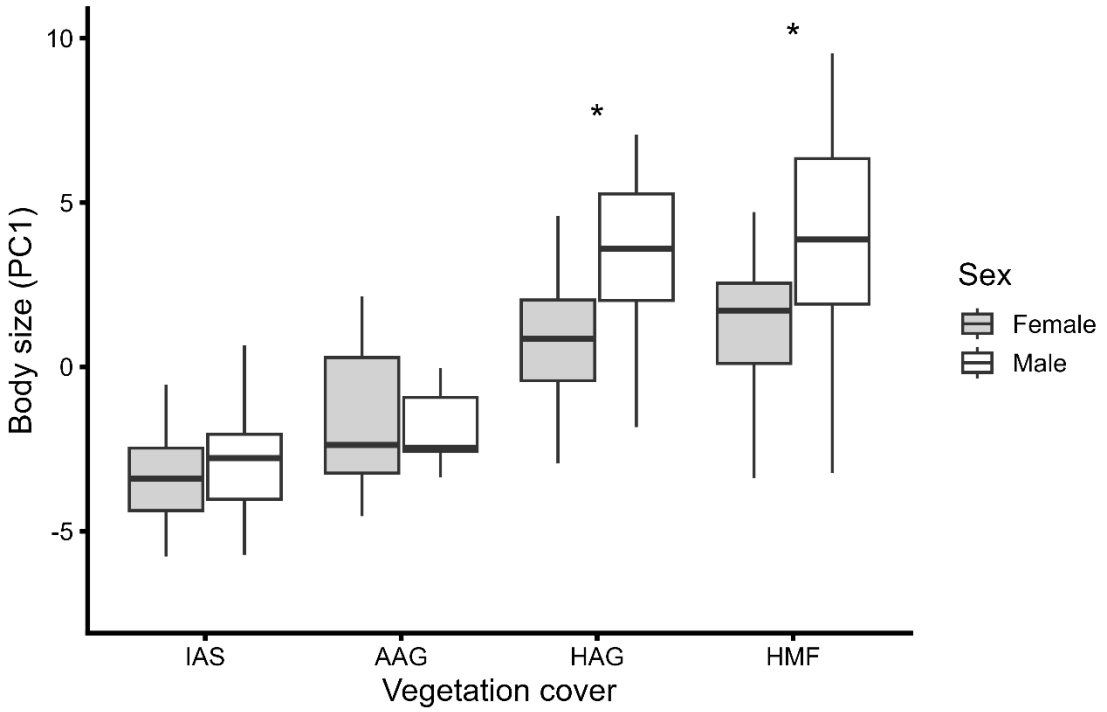


Figure 1.3 Relationship between (A) climatic moisture index (CMI), (B) temperature seasonality (BIO4) and sex adjusted body size in *Proctoporus lacertus*. Turquoise represents males, whereas salmon represents females.

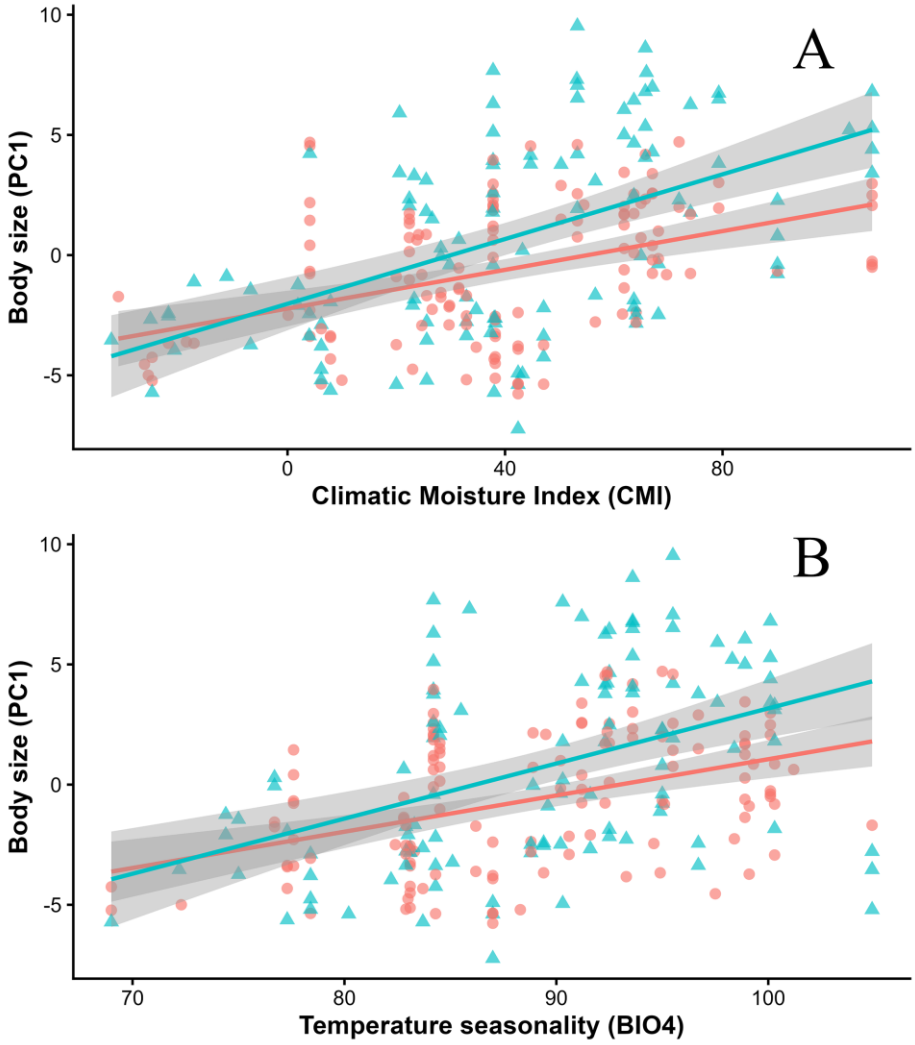


Figure 1.4 Standardized effect sizes of environmental predictors explaining body size variation in *Proctoporus lacertus* under two linear mixed-effects model sets. Points represent estimated regression coefficients (β), and horizontal bars indicate 95% confidence intervals. Asterisks indicate statistical significance: $p < 0.05$, $p < 0.01$, $p < 0.001$.

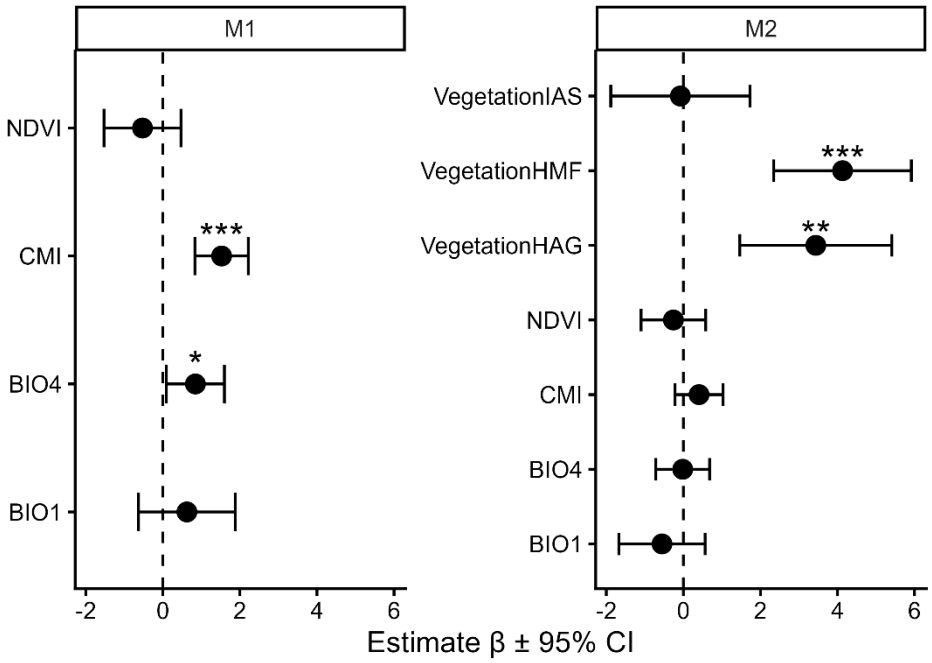


Table 1.1 Robust post-hoc comparisons among vegetation types by sex. HMF, humid montane forest; HAG, humid Andean grassland; AAG, arid Andean grassland; IAS, inter-Andean shrublands.

Contrast (sex)	Estimate	SE	df	t	*p*
IAS – AAS (♀)	-1.896	0.756	231	-2.51	0.061
IAS – HAG (♀)	-4.354	0.569	231	-7.65	**<0.001**
IAS – HMF (♀)	-4.714	0.463	231	-10.18	**<0.001**
AAG – HAG (♀)	-2.457	0.816	231	-3.01	**0.015**
AAG – HMF (♀)	-2.818	0.747	231	-3.78	**0.001**
HAG – HMF (♀)	-0.36	0.557	231	-0.65	0.916
IAS – AAS (♂)	-1.123	0.868	231	-1.29	0.568
IAS – HAG (♂)	-6.526	0.595	231	-10.97	**<0.001**
IAS – HMF (♂)	-6.461	0.501	231	-12.89	**<0.001**
AAG – HAG (♂)	-5.404	0.925	231	-5.84	**<0.001**
AAG – HMF (♂)	-5.339	0.868	231	-6.15	**<0.001**
HAG – HMF (♂)	0.065	0.595	231	0.109	0.999

Table 1.2 Results of the lineal mixed-effects models (LMMs) evaluating the effects of ecological variables on body size variation under the two model structures. BIO1, annual mean temperature; BIO4, temperature seasonality; CMI, climatic moisture index; NDVI, normalized difference vegetation index; veg, vegetation cover. HMF, humid montane forest; HAG, humid Andean grassland; AAG, arid Andean grassland; IAS, inter-Andean shrublands.

Models	Predictor	Estimate β	Standard error	df	t	*p*
M1	Intercept	-0.933	0.35	82.71	-2.665	0.009
	Sex:Male	1.48	0.257	195.28	5.769	**<0.001**
	BIO1	0.583	0.673	79.09	0.866	0.389
	BIO4	0.893	0.397	68.83	2.25	**0.028**
	CMI	1.416	0.37	74.77	3.824	**<0.001**
	NDVI	-0.523	0.522	89.88	-1.002	0.319
M2	Intercept	−3.006	0.805	165	−3.734	**<0.001**
	Sex:Male	1.442	0.255	165	5.653	**<0.001**
	Vegetation:HAG	3.454	1.003	165	3.444	**<0.001**
	Vegetation:HMF	4.138	0.909	165	4.553	**<0.001**
	Vegetation:IAS	−0.070	0.924	65	−0.076	0.94
	BIO1	−0.551	0.578	65	−0.953	0.344
	BIO4	0.002	0.358	65	0.007	0.995
	CMI	0.387	0.32	65	1.21	0.231
	NDVI	−0.258	0.43	165	−0.601	0.549

Supplementary materials.

Table S1. Database of individuals used in this study, including museum voucher number, collection, elevation, sex, vegetation cover, PC1 scores, annual mean temperature (BIO1), temperature seasonality (BIO4), climatic moisture index (CMI), and normalized difference vegetation index (NDVI).

Figure S1.1 Variation in the dorsolateral and ventral coloration patterns of male *Proctoporus lacertus*. A, B, adult individual from an inter-Andean shrubland (2700 m a.s.l.); C, D, adult individual from arid Andean grassland (4200 m a.s.l.); E, F, adult individual from humid Andean grassland (4100 m a.s.l.).

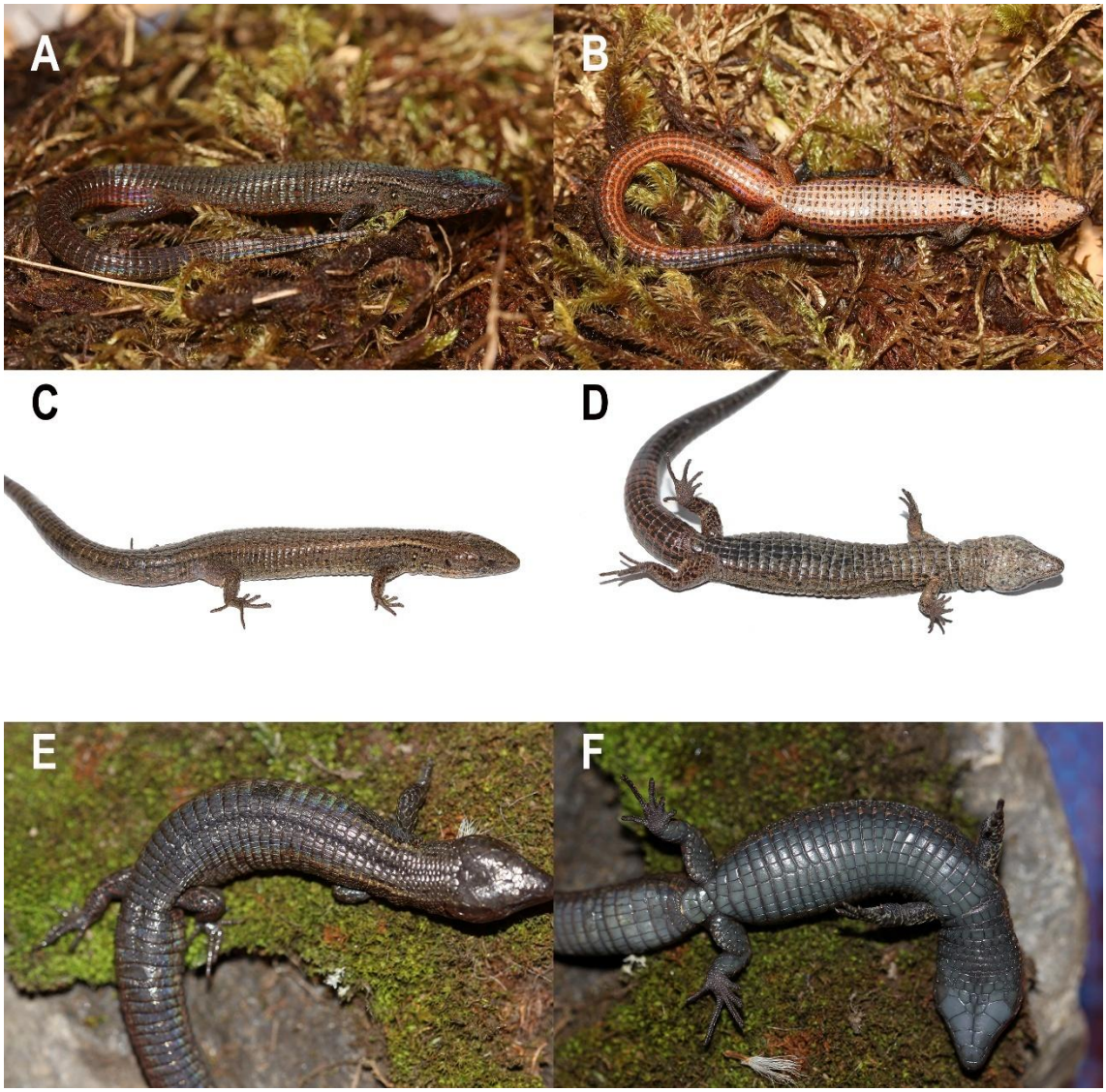


Figure S1.2 Geographic distribution of *Proctoporus lacertus* over vegetation cover (MINAM, 2015). Coloured points indicate the vegetation category associated with sampled individuals: yellow for humid Andean grassland (HAG), light blue for inter-Andean shrubland (IAS), red for arid Andean grassland (AAG) and light green for humid montane forest (HMF).

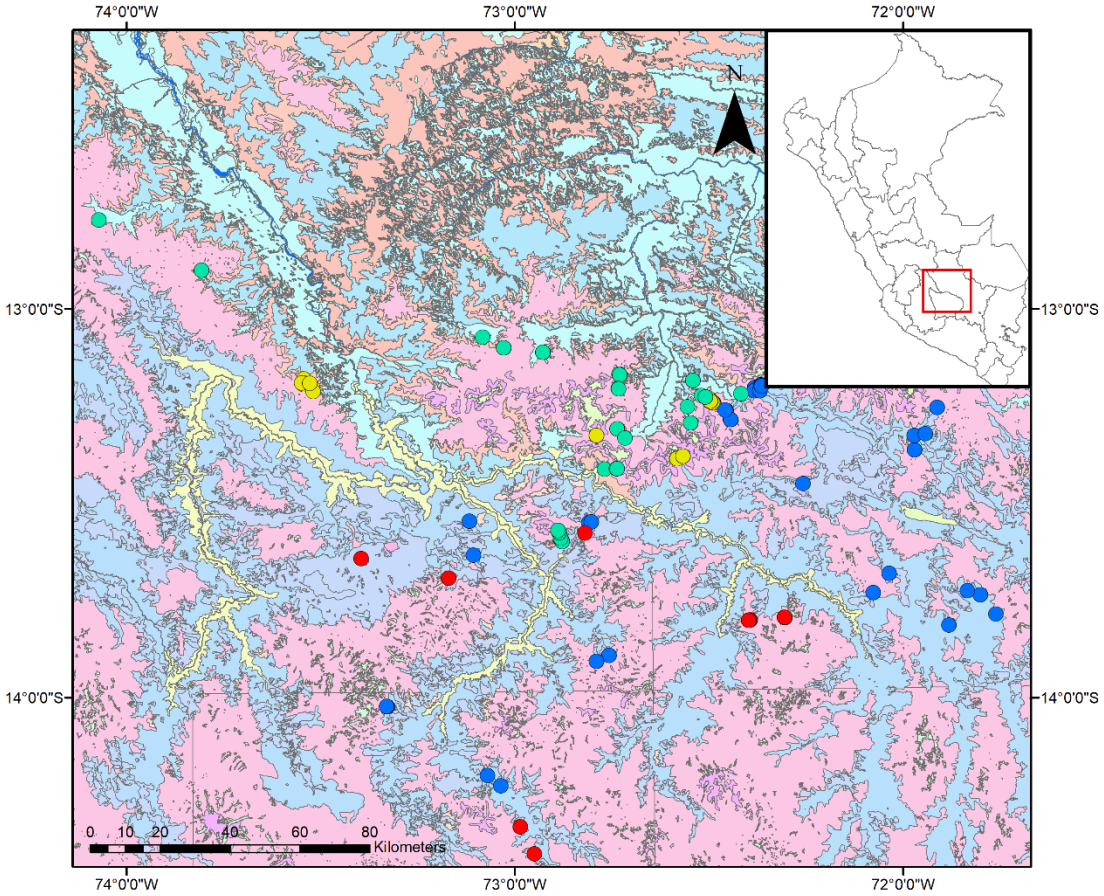
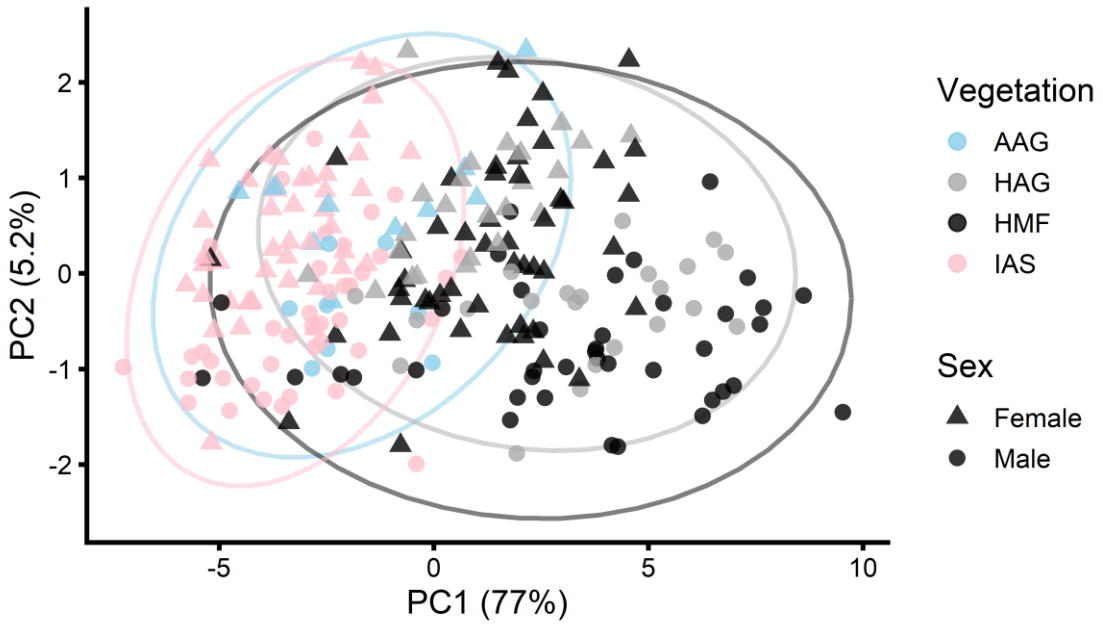


Figure S3 Principal component analysis of the 16 morphometric traits used as a body size index. HAG, humid Andean grassland; IAS, inter-Andean shrubland; AAG, arid Andean grassland; HMF, humid montane forest.



2. CAPÍTULO II: THERMAL ECOLOGY OF AN ANDEAN GYMNOPHTHALMID LIZARD ACROSS AN ALTITUDINAL GRADIENT IN THE PERUVIAN HIGHLANDS

2.1 ABSTRACT

Thermal ecology in ectotherms is strongly shaped by climatic heterogeneity, particularly in tropical mountains where environmental conditions change abruptly over short distances. Such gradients may promote broader thermal tolerances at high-elevation, although behavioral thermoregulation can buffer exposure to thermal extremes and reduce physiological divergence. We evaluated thermal traits, field body temperatures, operative temperatures, thermoregulatory effectiveness, and potential activity windows in the Andean gymnophthalmid lizard *Proctoporus lacertus* across an elevational gradient in the Peruvian Andes. The high-elevation population from Pacaymayu exhibited the broadest thermal tolerance, mainly because of a lower critical thermal minimum relative to the lower-elevation populations. In contrast, critical thermal maximum showed only weak differentiation, and selected temperature and voluntary upper thermal limits remained similar among localities. Voluntary lower thermal limits were also lower in Pacaymayu, producing a wider voluntary thermal range than in the lowest locality. Field data showed that body temperatures remained comparatively constrained despite broad and sometimes extreme operative temperatures, indicating strong behavioral and microhabitat buffering. Under-rock refuges provided the greatest potential activity time, whereas exposed microhabitats sharply restricted activity windows. Overall, our results suggest that thermal divergence in *P. lacertus* is expressed mainly at the cold end of the thermal niche, while behavioral thermoregulation and refuge use buffer exposure to extreme operative temperatures along the elevational gradient.

Keywords: Andean lizard; ectotherms; thermal ecology; gymnophthalmid lizard; elevational gradient; thermoregulation.

2.2 INTRODUCTION

Body temperature directly constrains physiological function in ectotherms because it influences the rate of biochemical reactions, the stability of proteins and membranes, and water balance (Angilletta Jr., 2009; Moyes & Schulte, 2014). Consequently, even small thermal changes can alter metabolic rate, enzymatic activity, locomotion, digestion, growth, and reproduction, ultimately affecting survival and reproductive success (Abram et al., 2017; Angilletta Jr., 2009; Bozinovic, 2015). Prolonged exposure to temperatures outside the tolerance range can also cause cellular damage. At high thermal extremes, heat stress may induce protein denaturation, disrupt enzymatic activity, and reorganize membrane phospholipids, thereby increasing mortality risk (Angilletta Jr., 2009; Pörtner & Farrell, 2008). Conversely, cold extremes can be lethal because of freezing risk and their negative effects on membrane fluidity and gas exchange, reducing ATP production and, in severe cases, causing cellular destruction through the formation of ice crystals (Angilletta Jr., 2009; Korner, 2021).

Elevational and latitudinal gradients therefore provide a natural framework for assessing how thermal regimes shape physiological and behavioral traits. At the macroclimatic scale, mean temperature generally decreases with elevation and latitude, but daily and seasonal thermal amplitudes vary depending on cloud cover, humidity, radiation, and topography, thereby determining the thermal mosaic available across microenvironments (Angilletta Jr., 2009). In tropical mountains, reduced seasonality has been proposed as a factor favoring narrower thermal tolerances and, consequently, stronger physiological barriers along elevational gradients (Ghalambor et al., 2006; Janzen, 1967; Sheldon et al., 2018). However, in high-elevation tropical mountains, the combination of intense daytime radiation and nocturnal cooling can generate pronounced daily thermal amplitudes, including nighttime temperatures below 0 °C, producing conditions

comparable to those found at higher latitudes (Janzen, 1967; Muñoz & Bodensteiner, 2019). This thermal heterogeneity along elevational gradients may impose selective pressures associated with both cold and heat, favoring populations with broader thermal breadths in high-elevation environments, in agreement with Janzen's climate variability hypothesis (Janzen, 1967).

Nevertheless, even if thermal heterogeneity promotes divergence in thermal tolerances, the extent of this divergence may be attenuated by behavioral thermoregulation. Behavioral thermoregulation buffers exposure to extreme conditions and reduces the strength of natural selection, producing evolutionary inertia, commonly referred to as the Bogert effect (Bogert, 1949; Huey et al., 2003; Muñoz, 2022). Under this scenario, ectotherms may maintain relatively similar body temperatures across climatic gradients by using vegetation cover and refuges, thereby weakening selective pressure for local physiological adaptation (Muñoz, 2022; Scheffers et al., 2014; Sunday et al., 2014). Consistent with this idea, studies along elevational and latitudinal gradients have shown that some thermal traits, particularly critical thermal minimum (CT_{min}), can diverge more rapidly than selected temperature (T_{sel}) and critical thermal maximum (CT_{max}) (Bozinovic et al., 2014; Muñoz & Bodensteiner, 2019; Sunday et al., 2019). A proposed mechanism underlying this pattern is the day–night asymmetry in thermoregulatory opportunities: during the day, thermal heterogeneity allows individuals to select suitable microhabitats and maintain body temperatures near their optimum, buffering directional selection for increased heat tolerance. At night, however, lower temperatures and reduced thermal heterogeneity limit behavioral compensation, increasing unavoidable exposure to cold and strengthening selection for improved cold tolerance. This pattern is known as Brett's hypothesis (Brett, 1956; Gaston et al., 2009).

Studies conducted in the tropical Andes have revealed recurrent, although not universal, patterns of thermal variation along elevational gradients. In amphibians, activity temperatures and critical thermal limits often decrease with elevation, with CT_{min} tending to be more labile than CT_{max} (Pintanel, 2019; Rueda Solano et al., 2016; von May et al., 2017). Similar asymmetries have been reported in several Andean reptiles, including *Anolis* and *Stenocercus*, where high-elevation populations frequently exhibit lower CT_{min} values while CT_{max} remains comparatively conserved (Guerra-Correa et al., 2026; Guerra-Correa et al., 2020; Méndez-Galeano et al., 2020; Pinzón-Barrera et al., 2024). However, this pattern is not universal: in *Liolaemus aparicioi*, body temperature decreases with elevation, but CT_{min}, CT_{max}, voluntary traits, and thermal breadth show little or no differentiation across a 1000-m gradient (Miranda-Calle et al., 2021). Thus, Andean ectotherms provide a useful comparative framework for testing whether elevational thermal divergence is expressed primarily through cold tolerance, through coordinated shifts in both thermal limits, or through behavioral buffering. In contrast, lizards of the genus *Proctoporus* remain poorly characterized in terms of their thermal ecology. Although CT_{max} has been reported for *P. unsaacae* and *P. lacertus* from localities at approximately 2740–2870 m elevation, other fundamental thermal traits remain unknown (Doan et al., 2022).

Although CT_{min} and CT_{max} are widely used to describe thresholds of physiological failure, they do not capture the temperatures at which animals begin to behaviorally avoid thermal stress. Then, selected temperatures, voluntary limits, and critical limits represent different levels of thermal response: preferred thermal states, behavioral withdrawal thresholds, and physiological failure points, respectively. Accordingly, voluntary thermal maximum (VT_{max}) and voluntary thermal minimum (VT_{min}) provide complementary information by identifying the temperatures at which individuals abandon activities such

as foraging or reproduction to seek refuge and avoid excessive heat or cold (Taylor et al., 2021). However, these variables remain rarely evaluated, especially in Andean reptiles. Therefore, integrating critical limits, voluntary limits, selected temperatures, and microhabitat-scale thermal availability is essential for interpreting ecological constraints and climatic vulnerability. Here, we evaluated the thermal ecology of *Proctoporus lacertus* across an elevational gradient in the Peruvian Andes by integrating laboratory estimates of selected temperature, voluntary thermal limits, critical thermal limits, and thermal breadths with field estimates of body temperature, operative temperature, thermoregulatory precision, and microhabitat-scale thermal availability. This design allowed us to test predictions derived from Janzen's climate variability hypothesis, the Bogert effect, and Brett's hypothesis. Specifically, we predicted that: (1) CT_{max} would show relative invariance across localities, whereas CT_{min} would decrease at higher elevations; (2) thermal tolerance breadth would be greater at higher elevations, where thermal variability is more pronounced; and (3) selected temperatures and voluntary thermal limits would remain comparatively conserved along the gradient if behavioral thermoregulation buffers exposure to environmental variation. Finally, we estimated potential survival and activity hours across microhabitats to evaluate how thermal availability constrains ecologically usable time under field conditions.

2.3 MATERIALS Y METHODS

Species

Proctoporus lacertus is a small-bodied lizard that exhibits geographic variation in body size along ecological gradients (Doan & Castoe, 2003; Mamani et al., 2026). Individuals from more humid areas tend to be larger, whereas those from arid areas are smaller

(Mamani et al., 2026*)*. This species is characterized by philopatry, and it has been suggested that *P. lacertus* may behave as a thermoconformer (Doan et al., 2021). In addition, previous studies have reported high critical thermal maxima for this species, reaching up to 39.4 °C, which has been interpreted as evidence of low vulnerability to the direct effects of global warming (Doan et al., 2022)

Study area and sampling

The study area was located within the Historic Sanctuary of Machu Picchu and its buffer zone, in Cusco, Peru (Fig. 1). We selected four localities along an elevational gradient of approximately 1000 m: Pacaymayu, the highest-elevation locality; Paucarcancha and Wayllabamba, representing intermediate elevations; and Pikillacta, the lowest-elevation locality. Pacaymayu corresponds to a high-Andean environment dominated by humid grasslands and wet puna vegetation, characterized by low temperatures, high atmospheric humidity, and reduced evapotranspiration rates (Oliveras et al., 2014). In contrast, Paucarcancha, Wayllabamba, and Pikillacta are located in inter-Andean valleys characterized by more xerophytic vegetation and apparently warmer climatic conditions, with higher solar radiation, seasonal water deficit, and elevated evapotranspiration rates (Silva et al., 2008).

We obtained adult individuals of *Proctoporus lacertus* from: (i) Pacaymayu (13°14'20.38"S, 72°29'30.25"W; 3860 m a.s.l.), (ii) the surroundings of the Paucarcancha archaeological complex (13°16'53.88"S, 72°26'52.63"W; 3200 m a.s.l.), and (iii) the surroundings of the Pikillacta archaeological complex (13°12'34.57"S, 72°22'8.60"W; 2800 m a.s.l.) (Fig. 1). The fieldwork was carried out between 20 and 27 August 2024.

Thermal traits

To evaluate lizard thermal traits, we conducted common-garden experiments in the city of Cusco, at 3200 m a.s.l. To minimize variation attributable to prior environmental conditions and standardize physiological state, individuals were kept in captivity for approximately four weeks before measurements, with continuous access to water and food. We confirmed that individuals had fed two days before each trial to reduce variation associated with energetic conditions. Laboratory trials began on 17 September 2024.

Selected temperature (T_{sel}) was estimated using a linear thermal gradient based on the method proposed by Hertz et al. (1993), in which an individual actively selects its preferred temperature. The gradient was constructed on a melamine base (1.0 × 0.33 m) with four parallel lanes, allowing four individuals to be tested simultaneously. The gradient was generated by placing a metal container (30 × 20 cm) with an ice block at one end as the cooling source and a continuous ceramic heat lamp at the opposite end as the heating source, producing an approximate thermal range of 5–45 °C. Given the semifossorial nature of the species, the lanes were covered to reduce visual disturbance and minimize observer influence on the individuals' thermal selection behavior. Each individual was placed in its lane at the beginning of the trial and allowed to move freely. Because of the small body size of the individuals, each lizard was allowed to acclimate to the thermal gradient for 35 min before T_{sel} was recorded. Following this acclimation period, cloacal temperature was measured rapidly using a four-channel thermocouple data logger (HOBO UX120-014M) equipped with type-T thermocouple probes (HOBO; accuracy ±0.6 °C). The initial temperature of environment each trial was recorded with a temperature/relative humidity data logger (HOBO MX2301A).

We defined critical thermal maximum (CT_{max}) as the temperature at which individuals showed the onset of muscle spasms (Doan et al., 2022). By contrast, VT_{max} was defined as the behavioral threshold at which the individual initiated evident activity and movement, interpreted as an avoidance response to overheating (Camacho & Rusch, 2017; Cowles & Bogert, 1944; Taylor et al., 2021).

Measurements were performed in a thermal bath using a circular plastic container (42 cm diameter × 23 cm height), starting at 24 °C. Four cylindrical plastic jars were placed simultaneously inside the bath as individual containers (8 cm diameter × 10 cm height; 1.00 mm thickness) and fixed together with tape. Water temperature was gradually increased through standardized additions of hot water prepared with an electric kettle and stored in thermos flasks, with 250 mL of hot water added every 5 min. During heating, when VT_{max} was reached, cloacal temperature was immediately measured, and the individual was returned to its container. We then continued increasing the bath temperature through successive additions of hot water until 39 °C. When the individual reached CT_{max}, cloacal temperature was measured, and it was immediately returned to its respective terrarium. The heating rate of the bath was approximately 0.9 °C min^{-1}, and each trial lasted approximately 35–40 min.

We defined CT_{min} as the lower temperature at which an individual loses the righting reflex when placed in dorsal recumbency (Taylor et al., 2021), and VT_{min} as the temperature at which, from a resting state, the individual initiated evident movement and displacement, which we interpreted as an avoidance response before overcooling (Taylor et al., 2021). Measurements were performed using the same system described for CT_{max}, but bath temperature was reduced by adding ice cubes every 5 min, while water temperature was continuously monitored. During cooling, the first movement observed from rest was considered VT_{min}, and cloacal temperature was immediately recorded. Temperature was

subsequently decreased to values near 1.0 °C, and the righting reflex was assessed periodically. When individuals reached CT_{min}, cloacal temperature was measured, and it was immediately returned to its respective terrarium. The cooling rate was approximately 1.0 °C min^{-1}, and each trial lasted approximately 40–50 min.

We followed a standardized experimental sequence in which T_{sel} was estimated first, followed by VT_{min} and CT_{min}, and finally VT_{max} and CT_{max}. Individuals were allowed to recover for five days between trials before subsequent measurements were conducted.

Thermoregulatory precision and calibration of operative models

We estimated thermoregulatory precision in the two thermally contrasting localities, Pacaymayu and Pikillacta, following the framework of Hertz et al. (1993), by integrating field body temperatures (T_b), operative temperatures (T_e), and the selected temperature range (T_{sel}). Operative temperatures were quantified using copper models (9 mm diameter × 60 mm length), painted dark brown to approximate the dorsal coloration of *Proctoporus lacertus*.

The copper models were calibrated in the laboratory using immobilized males from Pacaymayu and Pikillacta. Each model was sealed and fitted with a type-T thermocouple probe wrapped in moistened cotton and connected to a data logger (HOBO UX120-014M). Model temperatures were calibrated against lizard body temperatures under gradual heating with a ceramic lamp, showing strong linear relationships ($R^2 = 0.998$ and 0.979) and confirming that copper models reliably approximated body temperatures. Because calibration curves were highly linear in both contrasting localities, the same model configuration was used to estimate operative temperatures across all sampled microhabitats, including Wayllabamba.

The models were then deployed in Pacaymayu, Wayllabamba, and Pikillacta. At each locality, we selected five representative microhabitats: among tall grasses, under rocks, under bushes, on rocks, and on grasses. T_e values were recorded every 10 min over a 24-h period. In parallel, we recorded cloacal T_b of adult individuals during the activity period (10:00–14:00 h). Fieldwork to estimate T_e was conducted in June 2025: 19–20 in Pacaymayu, 22 in Wayllabamba, and 24–25 in Pikillacta. Body temperatures were measured on 19–20 June in Pacaymayu and 24–25 June in Pikillacta. No adult individuals were recorded in Wayllabamba.

Using T_{sel}, T_b, and T_e data recorded between 10:00 and 14:00 h, we defined the selected temperature range (T_{set}) as the interval between the 25th and 75th percentiles of T_{sel}. We then calculated the absolute deviation of T_b from T_{set} as an estimator of thermoregulatory precision (*db*), and the absolute deviation of T_e from T_{set} as an index of habitat thermal quality (*de*). Finally, we estimated thermoregulatory effectiveness as $E = 1 - (db/de)$ (Hertz et al., 1993)

Thermal availability for survival and activity

For each microhabitat across the three localities, we used T_e values recorded by the models described above. Mean operative temperature (T_{em}) was used as an estimate of microenvironmental T_e and was calculated as the average of four thermocouples from continuous 24-h records, corresponding to 144 records per day. Based on T_{em}, we defined survival and activity windows as records in which T_{em} fell within CT_{min}–CT_{max} and VT_{min}–VT_{max}, respectively. For each record, T_{em} was classified as suitable or unsuitable for survival and activity, and available hours were estimated by summing the time intervals between consecutive suitable records (Table S1).

Statistical analysis

To evaluate differences in thermal traits among localities while accounting for body-size variation and the non-independence of individuals tested within experimental groups, we fitted separate ANCOVA-type linear mixed-effects models for T_{sel}, CT_{max}, CT_{min}, VT_{min}, VT_{max}, R_{tol}, and R_{vol}. Body mass was included as a continuous covariate, whereas sex and locality were included as fixed effects. Cooling-group identity was included as a random intercept in the CT_{min} and VT_{min} models, heating-group identity in the CT_{max} and VT_{max} models, and trial identity in the T_{sel} model. Cooling and heating group identities were included as crossed random intercepts in R_{tol} and R_{vol} models. Models were fitted using the *lmer* function in the *lme4* package (Bates et al., 2015). Fixed effects were evaluated using Type III F-tests with Satterthwaite-approximated denominator degrees of freedom in *lmerTest* (Kuznetsova et al., 2017). Pairwise comparisons among localities were based on estimated marginal means with Kenward–Roger degrees of freedom and Tukey-adjusted *p*-values in *emmeans* (Lenth & Piaskowski, 2025). Model assumptions were assessed using residual diagnostics for normality and homoscedasticity. Analyses were conducted in R (R Core Team, 2025), figures were generated using *ggplot2* (Wickham, 2016), and statistical significance was set at $p \leq 0.05$.

2.4 RESULTS

Temperatura de selección, límites térmicos voluntarios y críticos

We recorded T_{sel}, CT_{min}, CT_{max}, R_{tol}, and VT_{max} in 24 individuals from Pacaymayu, 11 from Paucarcancha, and 12 from Pikillacta. In turn, VT_{min} and R_{vol} were obtained for 17 individuals from Pacaymayu, 7 from Paucarcancha, and 9 from Pikillacta (Table 1).

We found differences in CT_{min} among localities ($F_{2,42} = 13.91$, $p < 0.001$). Tukey-adjusted pairwise comparisons showed that Pacaymayu had lower CT_{min} than Pikillacta ($\Delta = -1.752 \pm 0.386$, $p = 0.001$) and Paucarcancha ($\Delta = -1.821 \pm 0.399$, $p = 0.001$), whereas Pikillacta and Paucarcancha did not differ ($p > 0.97$). R_{tol} also differed among localities ($F_{2,9.06} = 14.18$, $p = 0.001$), and Tukey-adjusted pairwise comparisons showed higher values in Pacaymayu than in Pikillacta ($\Delta = 2.40 \pm 0.55$, $p = 0.004$) and Paucarcancha ($\Delta = 2.65 \pm 0.57$, $p = 0.002$) and whereas Paucarcancha and Pikillacta did not differ ($p = 0.872$). In contrast, CT_{max} did not differ among localities ($F_{2,10.34} = 2.63$, $p = 0.12$) (Tables 2, 3; Fig. 2).

Among voluntary thermal traits, VT_{min} differed among localities ($F_{2,9.62} = 5.0$, $p = 0.032$), with Tukey-adjusted pairwise comparisons showing lower values in Pacaymayu than in Pikillacta ($\Delta = -3.14 \pm 1.0$, $p < 0.025$), whereas the remaining contrasts were not significant ($p > 0.196$). In contrast, T_{sel} ($F_{2, 11.26} = 0.56$, $p = 0.589$), VT_{max} ($F_{2, 11.48} = 0.44$, $p = 0.657$), and Rvol ($F_{2, 7.5} = 3.45$, $p = 0.087$) did not differ among localities (Tables 2, 3; Fig. 3).

Body temperature, operative temperature, and thermoregulatory precision

We recorded T_b from 20 adult individuals (11 males and 9 females) from Pacaymayu, and 18 individuals (12 males and 6 females) from Pikillacta. In Pacaymayu, T_b ranged from

8.11 to 24.48 °C, with a mean of 18.71 °C, whereas in Pikillacta it ranged from 14.45 to 20.94 °C, with a mean of 17.16 °C (Table 4). Operative temperatures varied widely in both localities, with extreme values mainly associated with exposed microhabitats. In Pacaymayu, T_e ranged from 9.64 to 68.39 °C, with a mean of 29.59 °C, whereas in Pikillacta it ranged from 9.37 to 52.57 °C, with a mean of 21.04 °C (Table 4).

Hertz indices indicated differences in thermoregulatory performance between localities. In Pacaymayu, *db* was lower (4.12 °C) than in Pikillacta (5.35 °C), indicating greater precision in Pacaymayu. By contrast, in Pacaymayu *de* was higher (11.59 °C) compared with Pikillacta (8.17 °C), consistent with a more thermally heterogeneous environment or greater deviation from the selected temperature range. Thermoregulatory effectiveness (*E*) was higher in Pacaymayu (0.64) than in Pikillacta (0.345), suggesting that despite lower habitat thermal quality, individuals in Pacaymayu maintained their T_b relatively closer to the preferred range than individuals in Pikillacta (Table 4).

Thermal characteristics of microenvironments and activity hours

In Pacaymayu, T_e showed its widest range in exposed microhabitats, where models recorded temperatures from −2.24 to 68.40 °C. In contrast, shaded microhabitats showed more restricted thermal ranges; under shrubs, T_e ranged from 2.2 to 14.72 °C. In terms of thermal availability, the under-rock microhabitat provided the greatest number of activity hours on both days, 9.33 h and 11.0 h, followed by the tall-grass microhabitats (7.67 h and 8.33 h). Exposed microhabitats, such as on rocks and on grasses, reduced activity time to 4.5–4.83 h and 2.0–2.83 h, respectively. The under-bushes microhabitat provided the fewest activity hours, 2.67 h and 4.67 h, despite maintaining relatively broad survival windows, up to 12 h on 20 June (Table S1; Fig. 4).

In Wayllabamba, T_e in exposed microhabitats ranged from 0 to 33.16 °C on grasses and from 1.34 to 32.45 °C on rocks. Survival hours were relatively homogeneous across microhabitats, ranging from 6.5 to 8.5 h. In contrast, activity hours were low to intermediate, ranging from 1.33 to 4.50 h, with the lowest value recorded under rocks, 1.33 h. The highest activity times were recorded in open microhabitats, such as on grasses and on rocks, with 4.3 and 4.50 h, respectively (Table S1; Fig. 4).

In Pikillacta, T_e minimum values were higher than in Pacaymayu, ranging from 1.15 to 9.63 °C, but exposed microhabitats also reached high maximum temperatures, up to 52.57 and 46.59 °C on grasses. Survival hours were consistently high in most microhabitats, ranging from 13.67 to 24 h. However, activity hours remained limited and strongly dependent on microhabitat. The under-rocks provided the greatest activity time, 4.83 h and 5.17 h, whereas exposed microhabitats, such as on rocks and on grasses, restricted activity to 1.33–3.17 h. The most extreme case occurred under bushes on the second evaluation day, with only 0.17 h of activity despite 24 h of survival (Table S1; Fig. 4).

2.5 DISCUSSION

Our results show that thermal divergence in *Proctoporus lacertus* along the elevational gradient is expressed mainly at the cold end of the thermal niche. The high-elevation population from Pacaymayu exhibited lower CT_{min} and broader thermal tolerance than the two lower-elevation populations, whereas CT_{max} remained conserved across localities. This pattern supports an asymmetry between lower and upper thermal limits, consistent with Brett's hypothesis (Brett, 1956; Gaston et al., 2009), and suggests that cold tolerance is a more labile component of thermal physiology in this Andean lizard. Similar patterns have been reported along elevational and latitudinal gradients, where CT_{min} often varies more strongly than CT_{max} (Bozinovic et al., 2014; Muñoz &

Bodensteiner, 2019; Sunday et al., 2019). In lizards of the genera *Stenocercus* and *Anolis*, the high-elevation populations exhibit lower CT_{min} values without equivalent changes in CT_{max} (Guerra-Correa et al., 2026; Guerra-Correa et al., 2020; Méndez-Galeano et al., 2020). However, Andean evidence is not uniform. In some *Anolis* lizards, CT_{min} has been reported to decrease with elevation, but CT_{max} also decreases in parallel, such that the shift toward colder environments does not necessarily result in greater cold–heat asymmetry, but rather in a joint displacement of both limits (Salazar et al., 2025); and in *Liolaemus aparicioi*, thermal traits remain unchanged across 1000 m of elevation (Miranda-Calle et al., 2021).

This asymmetry can be mechanistically explained by differences in thermoregulatory opportunities between day and night (Muñoz & Bodensteiner, 2019; Scheffers et al., 2014). During the day, microclimatic heterogeneity among microhabitats creates a thermal mosaic that allows behavioral compensation during the active period, reducing effective exposure to thermal extremes even when exposed microhabitats reach high T_e values. This buffering may weaken selection on CT_{max} and voluntary thermal thresholds, helping explain why some voluntary traits remain relatively conserved along the gradient. In contrast, during the night and cold periods, temperatures tend to become more homogeneous and cold exposure becomes largely unavoidable, consistent with our T_e records across the three localities evaluated (Fig. 4). In Pacaymayu, nocturnal T_e minima declined below 0 °C in all microhabitats, whereas daytime maxima in open habitats reached extremely high values, up to 68.4 °C. In Wayllabamba, daytime maximum T_e values were more moderate, but at night they fell below CT_{min} and approached 0 °C. In Pikillacta, minimum T_e values were generally higher and did not reach freezing point, although exposed microhabitats exceeded CT_{max} during the day and, on some nights, declined below CT_{min}. This day–night contrast may explain why selection acts more

strongly on the cold end of the thermal niche. In this context, it is useful to consider that extreme tolerance strategies occur in high-mountain environments: above 5000 m a.s.l., the frog *Pleurodema marmoratum* can recover after freezing at −3.036 °C for 6 h and tolerate temperatures up to 32.5 °C (Reider et al., 2021). It has also been reported that the lizard *Abronia taeniata* can tolerate freezing even when current operative temperatures in its habitat do not fall below 0 °C (Fierro-Estrada et al., 2023). However, because freeze tolerance was not directly assessed, subzero nocturnal temperatures in Pacaymayu suggest possible selective pressure from extreme cooling, but do not demonstrate physiological tolerance to freezing.

A previous study by Doan et al. (2022) reported a mean CT_{max} of 39.4 °C for *Proctoporus lacertus* from a locality approximately 15 km southeast of Pikillacta, at 2740 m a.s.l. In our study, we obtained a lower CT_{max} of 34.3 °C, this 5°C discrepancy suggests that CT_{max} estimates in *P. lacertus* may be sensitive to the methodological approach or experimental protocol. Our study used a four-week acclimation period and recorded temperature using a cloacal probe, whereas Doan et al. (2022) used a 16–24 h acclimation period and estimated temperature with an infrared thermometer. In fact, at the beginning of the CT_{max} trials, one individual reached 35.7 °C and did not survive. Therefore, we temporarily suspended the trials. We subsequently compared both thermal measurement methods and found that an infrared thermometer (Nubee-NUBB500H) recorded temperatures approximately 3.0 °C higher than cloacal measurements. Although Doan et al. (2022) did not report the brand or model of the infrared thermometer used, this difference suggests that part of their higher CT_{max} estimate could be explained by overestimation associated with infrared measurements. In addition, previous studies indicate that acclimation temperature and regime can modify CT_{max}, with increases of up to 4.0 °C in larvae of the toad *Rhinella horribilis* under warmer and fluctuating

acclimation conditions (Turriago et al., 2023), and increases of up to 2.2 °C in the lizard *Petrosaurus mearnsi* and 1.9 °C in *Anolis distichus* (Carilo Filho et al., 2022). However, acclimation effects alone are unlikely to account for the entire discrepancy. Seasonality could also influence estimates of thermal traits, but Doan et al. (2022) conducted their assessments in January, during the rainy and daily cool season, whereas our measurements were conducted in August–September, during a drier and daily warmer period. Thus, discrepancy likely reflects a combination of methodological and acclimation-related differences rather than a simple biological difference among populations. In any case, a 5 °C difference is biologically substantial and could affect estimates of thermal safety margins and inferred vulnerability to climate change.

The prediction of greater thermal breadth at higher elevation was supported by our results and is consistent with Janzen's hypothesis. Pacaymayu exhibited higher R_{tol} than Paucarcancha and Pikillacta, which did not differ from each other. This pattern matches the more variable microclimatic regime recorded at the highest elevation, characterized by lower minima and higher maxima (Fig. 4; Table S1). However, in *P. lacertus*, broader thermal breadth did not result from a symmetrical expansion of both limits, but mainly from increased cold tolerance at high elevation. Similar patterns have been documented across elevational gradients, although the relative contribution of lower and upper thermal limits varies among systems. In invertebrates from tropical gradients, Shah et al. (2017) found that elevational variation was expressed mainly through a reduction in CT_{min}, whereas CT_{max} changed little, so thermal breadth was dominated by the cold end. In Andean lizards of the genus *Anolis*, Salazar et al. (2025) reported that thermal tolerance range may remain relatively constant along elevation because CT_{min} and CT_{max} decrease in parallel. Nevertheless, other montane lineages exhibit broad or even broader tolerance ranges, such as *Stenocercus*, with higher values in high-elevation populations (Guerra-

Correa et al., 2020). Overall, our results and the available evidence indicate that the relationship between elevation and thermal breadth does not follow a single pattern but is strongly modulated by lineage and natural history.

Our results for voluntary traits provide partial support for the Bogert effect. We found no differences in T_{sel}, VT_{max}, and R_{vol} among localities (Fig. 3), indicating conservatism in most voluntary thermal traits. This pattern is consistent with the hypothesis that behavioral thermoregulation buffers individuals from environmental thermal variation, potentially reducing divergent selection on thermal preference and upper avoidance thresholds. Individuals inhabiting contrasting environments may therefore maintain similar body temperatures by selecting suitable microhabitats or adjusting their activity periods, thereby promoting evolutionary stasis across ecological gradients (Bogert, 1949; Huey et al., 2003). However, VT_{min} was lower at the high-elevation locality, indicating that the lower voluntary thermal threshold may be more responsive to cold conditions, similarly to CT_{min}. This shift may allow individuals from Pacaymayu to remain active at lower temperatures or to delay behavioral withdrawal during cooling without increasing the overall breadth of the voluntary thermal range (Fig. 3). This interpretation is consistent with the idea that voluntary thresholds reflect behavioral decisions that precede physiological failure and may be adjusted to maximize usable activity time under thermally restrictive conditions (Camacho & Rusch, 2017).

A central methodological issue concerns how VT_{min} and VT_{max} are operationalized. In our study, all individuals exhibited an unequivocal behavioral response to increasing temperature, which we interpreted as VT_{max}. However, different approaches have been used in literature. Some studies have estimated VT_{max} as the maximum value of T_{sel} (Jiménez-Robles & De la Riva, 2019), whereas others define it as the temperature at which an individual actively attempts to avoid the heat source, either by escaping or

abandoning the hot microhabitat (Chukwuka et al., 2020). Our data show that T_{sel} and VT_{max} do not overlap, suggesting that using the maximum T_{sel} as a proxy for VT_{max} may underestimate this threshold and artificially compress the voluntary thermal range toward lower temperatures. By contrast, defining VT_{max} based on active avoidance behavior may shift this threshold toward slightly higher temperatures. An analogous issue occurs at the lower thermal end: VT_{min} has also been defined as the minimum value of T_{sel} (Lara-Reséndiz et al., 2024), but in our study T_{sel} and VT_{min} also did not overlap, indicating that this approach may overestimate VT_{min} by shifting it toward higher temperatures. This distinction is conceptually important because T_{sel} does not necessarily coincide with either VT_{min} or VT_{max}. The former describes the distribution of temperatures selected in a thermal gradient, whereas the latter represents behavioral thresholds of avoidance. Although our method is simple and not necessarily free of bias, it allows these two levels of thermal response to be separated more clearly.

During the cooling experiment, we observed that approximately one-third of individuals did not display cold-avoidance behavior but instead remained without an evident response until approaching CT_{min} (Table 1). This suggests that, under progressive cooling, the cold end may not always be expressed as a well-defined behavioral threshold, because decreasing temperature gradually reduces activity and neuromuscular performance (Niu et al., 2021). Moreover, some individuals may cope with cooling through physiological mechanisms or through progressive depression of activity rather than behavioral avoidance. In freeze-tolerant vertebrates, the accumulation of cryoprotectants such as glucose, glycerol, or urea reduces cellular damage during exposure to subzero temperatures (Storey & Storey, 2017). Although we lack direct evidence for such mechanisms in *P. lacertus*, the limited VT_{min} response observed here is consistent with greater lability of lower thermal traits, because cold events can impose direct mortality

and favor local selective pressures that shift lower thermal limits toward greater cold tolerance (Sunday et al., 2019).

The evidence for a behavioral component is further reinforced by contrasting T_b and T_e. Although T_e reached extremely broad values in both localities, T_b recorded during the activity period remained within a relatively narrow range (Table 4), indicating that the environment offers potential thermal extremes, but that T_b is constrained through microhabitat selection and behavioral adjustments. These observations are consistent with Hertz indices; in Pacaymayu, the deviation of T_b from T_{set} was lower (db = 4.12 °C) than in Pikillacta (db = 5.35 °C), suggesting greater thermoregulatory precision at the highest-elevation locality. At the same time, the deviation of T_e from T_{set} was higher in Pacaymayu (de = 11.59 °C) than in Pikillacta (de = 8.17 °C), consistent with lower habitat thermal quality at higher elevation. Consequently, the combination of lower environmental thermal quality and greater thermoregulatory precision resulted in higher thermoregulatory effectiveness in Pacaymayu (E = 0.64) than in Pikillacta (E = 0.345). This suggests that, at the highest-elevation locality, thermoregulation is more necessary because the thermal environment deviates more strongly from the preferred range. Even under these conditions, individuals were able to maintain T_b relatively closer to their preferred range, probably by exploiting the microclimatic mosaic, which, although more variable among microhabitats, includes suitable thermal patches such as refuges under rocks and among tall grasses, where broader activity windows were concentrated (Tables 4, S1). These results contrast with the interpretation proposed by Doan et al. (2021), who suggested that *P. lacertus* is primarily thermoconforming and thigmothermic. Our data do not support strict thermoconformity. Rather, they are consistent with low to moderate thermoregulation, with relatively higher performance in Pacaymayu than Pikillacta. In addition, we observed that captive individuals exposed themselves to sunlight during the

morning, consistent with the opportunistic use of radiant energy as a possible mechanism to increase T_b.

Microhabitat-specific T_e records and estimates of potential survival and activity hours further link microhabitat use to microclimate and support the interpretation that *Proctoporus lacertus* exhibits semifossorial habits. Doan and Castoe (2003) and Doan et al. (2022) reported specimens under rocks, and our field observations are consistent with this pattern. We recorded individuals both under rocks and among tall grasses, precisely the microhabitats that provided the broadest windows of optimal temperatures for activity (Table 4; Fig. 4). This consistency suggests that semifossorial behavior does not merely reflect where individuals are found but rather represents a functional mechanism through which microhabitat selection maximizes the time available for activity by buffering thermal extremes. In Pacaymayu, exposed microhabitats reached extreme temperatures, up to 68.4 °C, which likely drastically reduces the time available for activity because of overheating risk, whereas subzero minima indicate cold-related constraints during the night. Even when relatively broad survival windows were available, activity hours were strongly determined by microhabitat (Table 4). The under-rock microhabitat provided the greatest thermal opportunity for activity, followed by areas among tall grasses, whereas under-bush microhabitats reduced activity to minimum levels. In Pikillacta, although survival potential was high in most microhabitats, activity remained limited and microhabitat-dependent, with the greatest opportunities under rocks and the lowest values under bushes.

Finally, the difference between survival and activity windows is especially relevant for interpreting climatic vulnerability. Even if warming does not cause direct mortality because CT_{max} is rarely exceeded in concealed microhabitats, it may increase refuge use and reduce the time available for foraging, reproduction, territorial defense, and

movement. Such reductions in activity time could have negative demographic consequences, as documented in other lizards (Sinervo et al., 2010). This vulnerability could be intensified by vegetation loss and habitat simplification, which reduce the availability of thermally buffered refuges and erode the microclimatic mosaic. Thus, in *P. lacertus*, climate change may affect populations primarily through a contraction of ecologically usable time rather than direct thermal mortality.

In conclusion, thermal divergence in *Proctoporus lacertus* across the Peruvian Andean gradient is expressed primarily through increased cold tolerance and broader thermal breadth at high elevation, rather than through marked shifts in upper thermal limits or selected temperature. At the same time, field body temperatures, Hertz indices, and microhabitat-specific activity windows indicate that behavioral thermoregulation and refuge use buffer exposure to extreme operative temperatures. These results support a view of Andean thermal adaptation in which physiological divergence and behavioral buffering act together, with the cold end of the thermal niche emerging as the most responsive component of intraspecific thermal variation.

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Author contributions

LM designed the study, conducted fieldwork, analyzed the data, and wrote the initial draft. **ERS**, **AC**, and **SSM** contributed to the study design, supervised the project, and critically reviewed the manuscript. **RC**, **EBH**, and **BDDM** assisted with field and laboratory data collection and contributed to the interpretation of the results. All authors reviewed and approved the final version of the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical considerations

The capture, handling, and temporary maintenance of individuals were conducted following procedures intended to minimize stress and risk of injury. Fieldwork, collection, and temporary holding of specimens for experimental purposes were carried out under research permits granted by the Servicio Nacional Forestal y de Fauna Silvestre

of Peru (RD No. D000125-2024-MIDAGRI-SERFOR-DGGSPFFS-DGSPFS) and the Santuario Histórico de Machu Picchu (No. 009-2024-SERNANP-SHM/J). At the end of the trials, individuals were returned to their respective sites of origin.

Figure 2.1 Location of the sampling sites: Pikillacta (1), Paucarcancha (2), Wayllbamba (3), and Pacaymayu (4). Thermal traits were measured at sites 1, 2, and 4; body temperature at sites 1 and 4; and operative temperature and activity/survival hours at sites 1, 3, and 4. The red line indicates the boundary of the Santuario Histórico de Machu Picchu. The photograph shows an adult male *Proctoporus lacertus* from Pacaymayu.

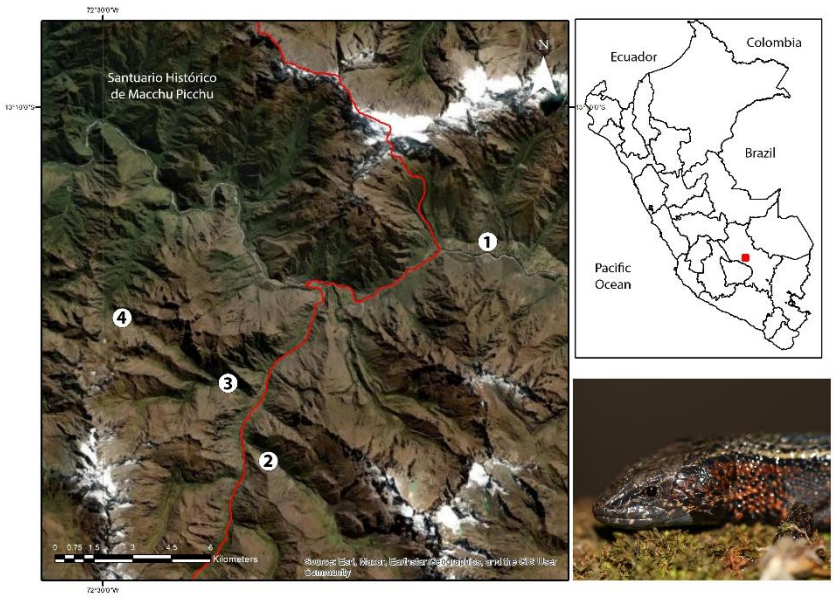


Figura 2.2 Thermal traits of *Proctoporus lacertus* by locality, including critical thermal maximum (CT_{max}), critical thermal minimum (CT_{min}), and thermal tolerance range (R_{tol}). The photograph shows a male from Paucarcancha.

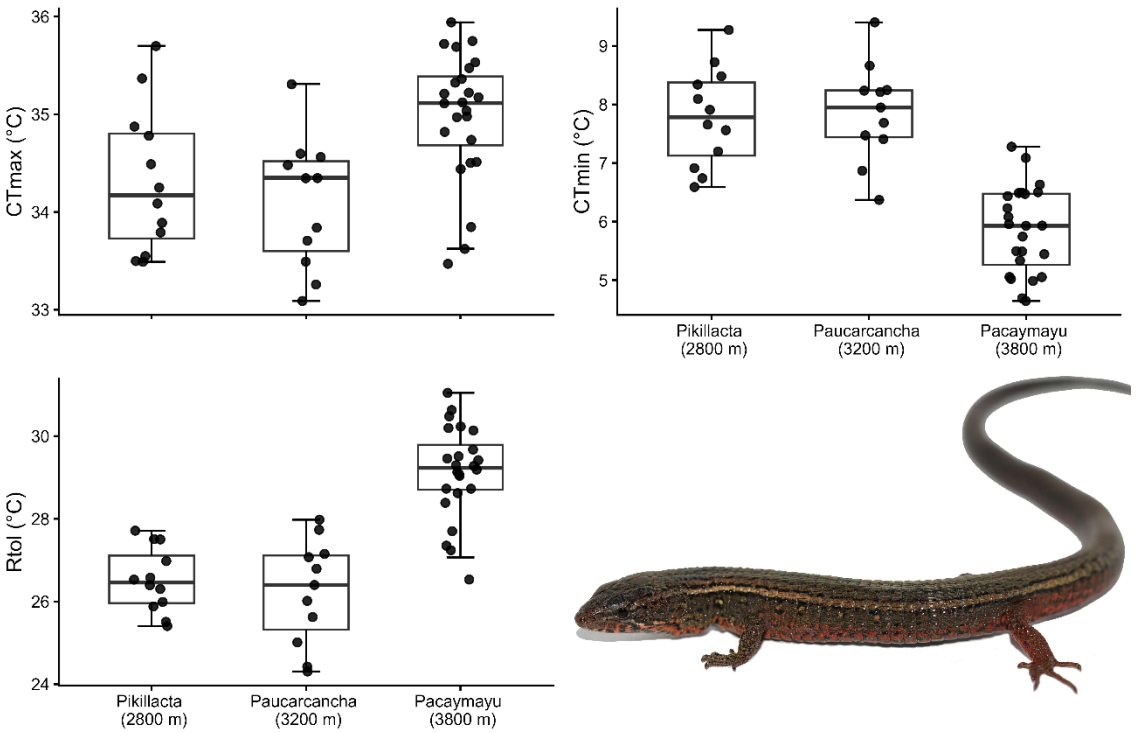


Figure 2.3 Voluntary thermal traits of *Proctoporus lacertus* by locality, including selected temperature (T_{sel}), voluntary thermal minimum (VT_{min}), voluntary thermal maximum (VT_{max}), and voluntary thermal range (R_{vol}).

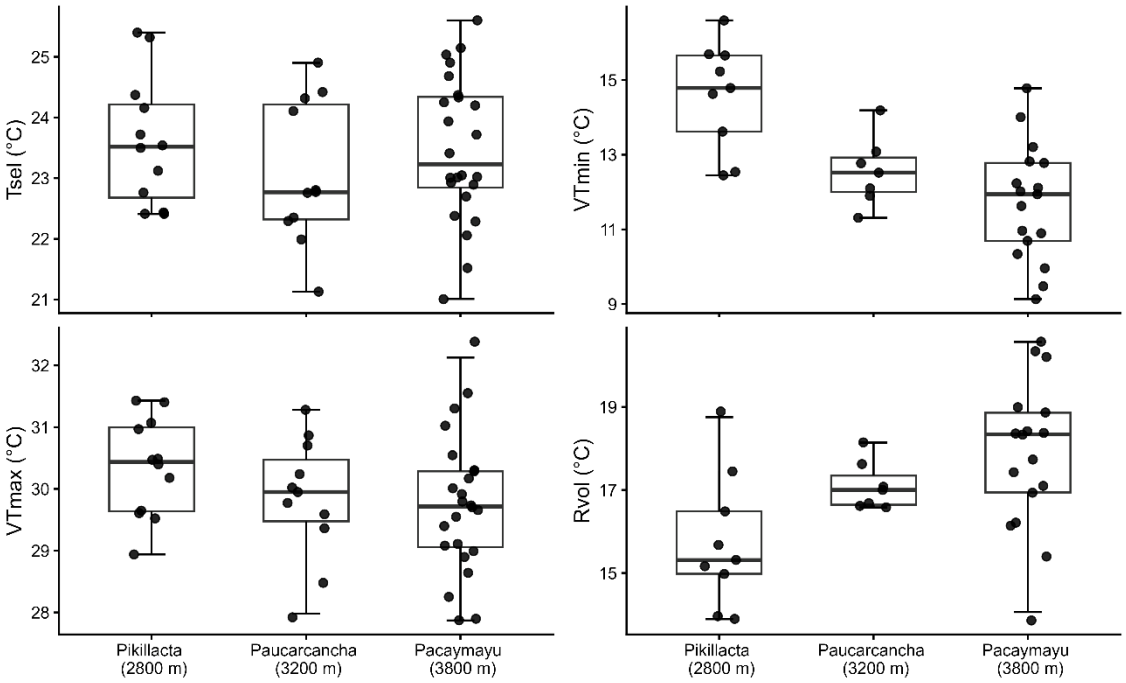
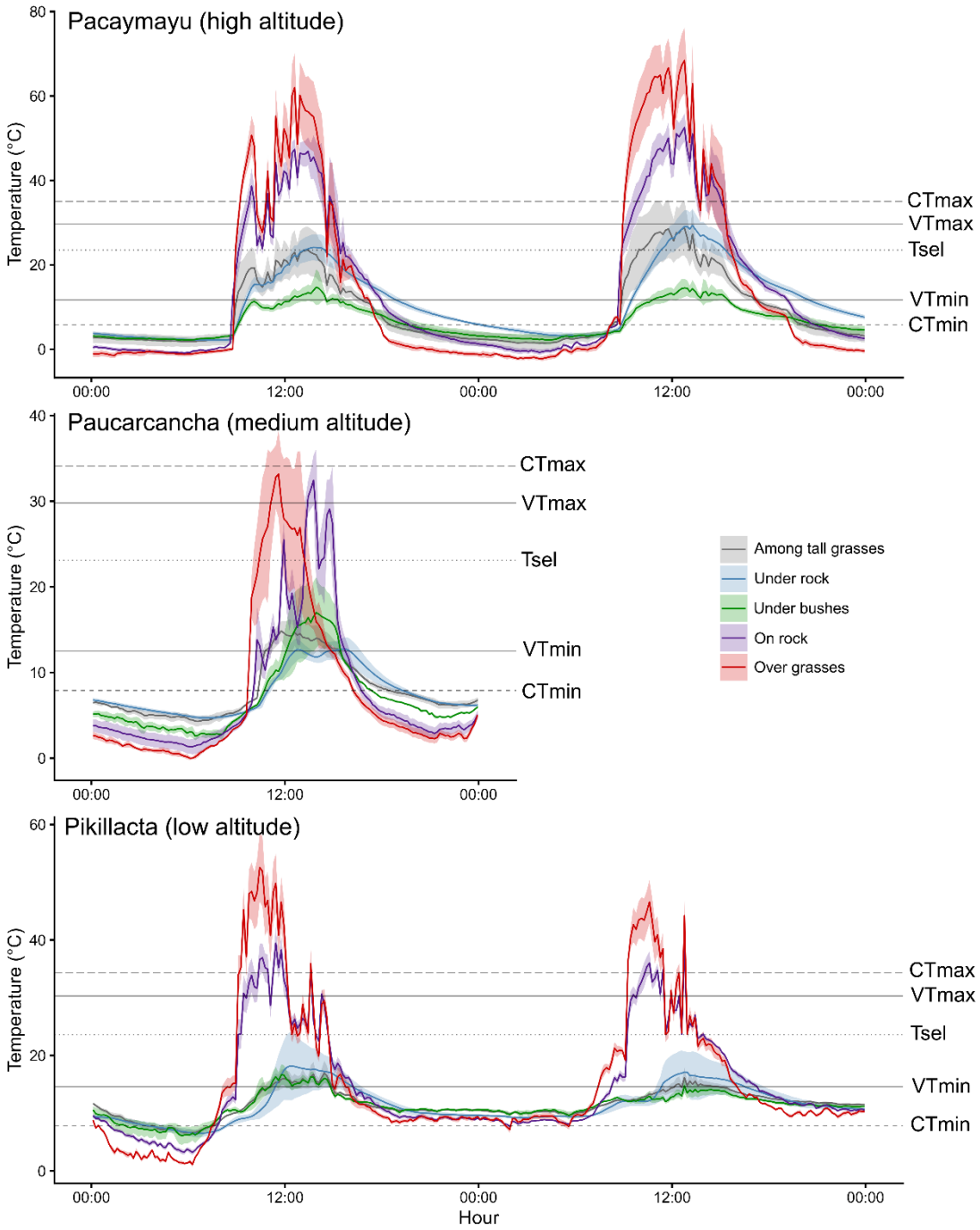


Figure 2.4 Daily 24-h fluctuation of operative temperature (Te) across five microhabitats in the three evaluated areas. Horizontal lines indicate the thermal traits of *Proctoporus lacertus* used to define survival and activity windows.



TABLES

Table 2.1 Thermal traits of *Proctoporus lacertus* across the three evaluated localities, reported as mean and observed range. Values are expressed in °C. N_2 indicates the number of individuals that showed a detectable behavioral response for estimating VT_{min}.

Locality (N)	N	Tsel	CTmin	CTmax	Rtol	VTmax	N	Vtmin	Rvol
Pacaymayu	24	23.48 (21.01–25.6)	5.85 (4.64–7.28)	34.98 (33.47–35.94)	29.12 (26.53–31.05)	29.75 (27.87–32.38)	17	11.71 (9.13–14.78)	17.84 (13.86–20.56)
Paucarcancha	11	23.07 (21.13–24.9)	7.86 (6.37–9.49)	34.09 (33.09–35.31)	26.23 (24.31–27.98)	29.83 (27.9–31.28)	7	12.55 (11.31–14.19)	17.1 (16.58–18.14)
Pikillacta	12	23.59 (22.41–25.4)	7.79 (6.59–9.27)	34.31 (33.49–35.7)	26.52 (25.41–27.71)	30.34 (28.94–31.43)	9	14.58 (12.45–16.6)	15.76 (13.89–18.89)

Table 2.2 ANCOVA results for thermal traits of *Proctoporus lacertus*, with body mass, sex, and locality included as explanatory variables.

Rasgo	Variables	df1	df2	F value	*p*
Tsel	Mass (g)	1	40.393	0.23	0.632
	Sex	1	39.216	2.54	0.119
	Locality	2	11.258	0.56	0.589
CTmin	Mass (g)	1	42	0.59	0.447
	Sex	1	42	0.00	0.982
	Locality	2	42	13.91	**<0.001**
CTmax	Mass (g)	1	37.432	0.08	0.782
	Sex	1	39.347	1.10	0.301
	Locality	2	10.341	2.63	0.120
Rtol	Mass (g)	1	33.014	0.40	0.530
	Sex	1	40.285	0.52	0.477
	Locality	2	9.065	14.18	**0.001**
VTmin	Mass (g)	1	24.759	0.84	0.369
	Sex	1	20.613	0.27	0.607
	Locality	2	9.624	5.00	**0.032**
VTmax	Mass (g)	1	39.702	0.76	0.388
	Sex	1	38.855	0.13	0.723
	Locality	2	11.479	0.44	0.657
Rvol	Mass (g)	1	24.041	1.83	0.188
	Sex	1	18.282	0.01	0.914
	Locality	2	7.504	3.45	0.087

Table 2.3 Tukey post hoc comparisons among localities based on estimated marginal means for thermal traits that differed significantly among the three localities.

Trait	Contrast	Estimate	SE	df	t	p
CT_{min}	Pacaymayu − Paucarcancha	−1.8217	0.399	13.89	−4.570	**0.001**
	Pacaymayu − Pikillacta	−1.7526	0.386	12.49	−4.542	**0.001**
	Paucarcancha − Pikillacta	0.0691	0.338	8.83	0.204	0.977
Rtol	Pacaymayu − Paucarcancha	2.651	0.575	11.07	4.608	**0.002**
	Pacaymayu − Pikillacta	2.404	0.555	9.51	4.328	**0.004**
	Paucarcancha − Pikillacta	−0.247	0.488	6.26	−0.505	0.872
VTmin	Pacaymayu − Paucarcancha	−1.08	1	10.5	−1.075	0.549
	Pacaymayu − Pikillacta	−3.14	1	10.31	−3.132	**0.025**
	Paucarcancha − Pikillacta	−2.06	1.09	8.55	−1.900	0.196

Table 2.4 Mean and range of field thermal variables and Hertz thermoregulation indices recorded between 10:00 and 14:00 h, including body temperature (T_b), operative temperature (T_e), thermoregulatory precision (*db*), habitat thermal quality (*de*), and thermoregulatory effectiveness (*E*).

Locality (N)	Tb(°C)	Te	*db*	*de*	*E*
Pacaymayu (20)	18.71 (8.11–24.48)	29.59 (9.64–68.39)	4.12	11.59	0.64
Pikillacta (18)	17.16 (14.45–20.94)	21.04 (9.37–52.57)	5.35	8.17	0.345

Supplementary material

Table S1 Potential survival and activity hours of *Proctoporus lacertus*, estimated from mean operative temperature (Tem) based on 24 h of continuous records. Minimum (T_{emin}) and maximum operative temperatures (T_{emax}) recorded at each locality are also reported.

Locality	Microhabitat	Day	N	T_{emin}	T_{emax}	Survival (h)	Activity (h)
Pacaymayu	Among tall grasses	1	144	1.85	23.45	9.67	7.67
	Under rocks	1	144	2.18	24.15	15	9.33
	Under bushes	1	144	2.2	14.72	10.5	2.67
	On rocks	1	144	-0.88	47.31	7.33	4.83
	On grasses	1	144	-1.44	62.01	4.67	2.83
	Among tall grasses	2	144	1.46	28.7	11.67	8.33
	Under rocks	2	144	3.14	29.34	15.17	11
	Under bushes	2	144	2.19	14.53	12	4.67
	On rocks	2	144	-0.72	52.5	7.33	4.5
	On grasses	2	144	-2.24	68.4	5.17	2
Wayllabamba	Among tall grasses	1	144	4.29	14.85	8.17	4.5
	Under rocks	1	144	4.69	12.74	8.5	1.33
	Under bushes	1	144	2.58	16.99	6.67	3.5
	On rocks	1	144	1.34	32.45	6.67	4.5
	On grasses	1	144	0	33.16	6.5	4.33
Pikillacta	Among tall grasses	1	144	6.65	16.96	20.17	3.67
	Under rocks	1	144	6.47	18.17	19.33	4.83
	Under bushes	1	144	6.09	16.65	18.83	3.83
	On rocks	1	144	3.22	39.45	15.83	1.33
	On grasses	1	144	1.15	52.57	13.67	1.33
	Among tall grasses	2	144	9.62	16.15	24	2.5
	Under rocks	2	144	9.24	17.14	24	5.17
	Under bushes	2	144	9.63	14.73	24	0.17
	On rocks	2	144	7.71	38.92	23	3.17
	Ov grasses	2	144	7.18	46.59	20.83	2.5

3. CAPÍTULO III: REDUCED BODY SIZE ASSOCIATED WITH ARIDITY CONSTRAINS COMPETITIVE ABILITY AND LIMITS RANGE EXPANSION IN AN ANDEAN LIZARD

3.1 ABSTRACT

Range limits correspond to the geographic edges where a species no longer maintains persistent populations. These edges may result from biotic and abiotic constraints that reduce species performance. In the Cordillera de Vilcabamba, *Proctoporus lacertus* inhabits areas ranging from high humid zones to arid inter-Andean valleys and reduces its body size toward more arid environments. In the Sacred Valley of the Incas, *P. lacertus* is altitudinally replaced by *P. cf. kiziriani*, a larger-bodied congener, showing an abrupt boundary potentially mediated by interspecific competition. In this context, we evaluated the hypothesis of a trade-off between body size reduction associated with arid environments and competitive ability, as a factor limiting the distribution range. We conducted two common-garden experiments with limited resources to evaluate the competitive performance of two morphotypes of *P. lacertus*: a small-bodied morphotype (*P. lacertus*-P) and a large-bodied morphotype (*P. lacertus*-G), both against *P. cf. kiziriani*. In each experiment, we used 12 individuals per taxon, distributed in six standardized cohabitation units (one male and one female per taxon in each unit), and recorded refuge use and body mass during the cohabitation period. The results showed that *P. cf. kiziriani* had a higher probability of refuge use and that males showed greater relative mass gain than *P. lacertus*-P. In contrast, *P. lacertus*-G had a higher probability of refuge use, and males maintained greater increases in mass than *P. cf. kiziriani*. Together, our results are consistent with competitive exclusion as a determining mechanism of the distribution range limit of both species

Keywords: Inter-Andean Valley, body size, competitive exclusion, ecological niche, limited resources.

3.2 INTRODUCTION

The ecological niche is defined as a multidimensional space that describes the set of conditions and resources under which a population can persist across generations (Hutchinson, 1957, 1978). Within this framework, climatic variables and biotic interactions (e.g., competition, predation and mutualisms) determine the capacity of species to survive, reproduce and sustain viable populations over time. In contrast, toward the margins of the niche, or outside it, environmental and ecological conditions reduce individual performance and population growth rate, preventing local persistence (Holt, 2009; Hutchinson, 1957; Soberon, 2007). Therefore, the niche, in any of its dimensions, is characterized by the fact that fitness is maximized within this space, whereas in the edge regions and outside the niche, it is considerably reduced (Hutchinson, 1957; Jaksic & Marone, 2007).

Classically, a distinction is made between the fundamental niche, understood as the hypervolume of conditions under which a species could persist in the absence of external constraints, and the realized niche, which corresponds to the set of conditions effectively occupied and is limited by factors such as dispersal, biotic interactions, biogeographic barriers, and other ecological and historical constraints (Carscadden et al., 2020; Holt, 2009; Soberon, 2007). Analogously, these concepts can be projected onto geographic space as the potential geographic range and the realized geographic range, although this projection depends on the spatial availability of suitable environments, the connectivity among them, and the biogeographic history of populations (Lomolino et al., 2017). Therefore, the size and shape of a species' geographic range depend both on the breadth of its niche and on the way in which the conditions that compose it are distributed and connected in geographic space.

The limits of species' distribution ranges are ubiquitous and constitute an active field of research (Gaston, 2009; Holt et al., 2022; Lyu & Alexander, 2022; Sexton et al., 2009; Willi & Van Buskirk, 2022). This interest has intensified in the context of global change, given that numerous species are modifying their historical ranges both altitudinally and latitudinally (Freeman et al., 2018; Parmesan, 2006). In this scenario, understanding the drivers of range dynamics is key to anticipating and mitigating their effects on biodiversity.

Multiple mechanisms operating at different levels have been proposed to explain the location of species' range limits. Among these, physiological constraints, such as thermal and hydric tolerances, are particularly important because abiotic stress can prevent populations from colonizing or persisting in challenging environments by reducing survival and reproductive success (Bozinovic, 2015; Cahill et al., 2014; Sunday et al., 2014). Biotic interactions, including competition, predation, parasitism and mutualisms, can also play a central role in setting distribution limits (Gaston, 2003; Godsoe et al., 2017; Pigot & Tobias, 2013). Thus, range limits rarely reflect a single cause, but instead emerge from the interaction among abiotic constraints, demographic processes, dispersal and local biotic interactions (Lomolino et al., 2017; Radomski, 2026).

Interspecific competition is recognized as a central mechanism shaping species' geographic ranges (Case et al., 2005; Godsoe et al., 2017; Price & Kirkpatrick, 2009), and can even delay colonization into novel environments (Legault et al., 2020). A relevant conceptual framework for understanding this process is the competitive exclusion principle, which predicts that when two species exploit similar and limited resources, long-term coexistence may be untenable if one species uses those resources more efficiently. In such cases, the competitively superior species may displace the other, ultimately leading to its local extinction (Holt, 2017; Levin, 1970). This mechanism may

be particularly relevant at range edges, where an expanding species may encounter both distinct environmental conditions and resident competitors that are better adjusted to the local environment. The importance of competitive exclusion in these contexts has been documented both in experiments under controlled conditions (Legault et al., 2020; Violle et al., 2010) and under natural conditions (Brown, 1971; Martin & Ghalambor, 2023).

Evolutionary compromises, or trade-offs, are another key factor shaping distribution ranges along ecological gradients. These trade-offs arise when improvement in one trait occurs at the expense of another because of constraints on time, energy or function, such that no phenotype can simultaneously maximize all components of fitness (Stearns, 1989). Consequently, an adaptive advantage in a particular environmental context may entail reduced performance under different conditions (Martin & Ghalambor, 2023; Willi & Van Buskirk, 2022). A classic example is the trade-off between abiotic stress tolerance and competitive ability. In this context, colonization of abiotically stressful environments may favour traits associated with greater physiological tolerance. However, these same traits may reduce competitive ability against resident species. As a result, the expansion of the colonizing species may be constrained by local biotic interactions (Hargreaves & Eckert, 2019; Martin & Ghalambor, 2023; Morin & Chuine, 2006).

However, the relative contribution of these factors may vary substantially depending on the type of range limit and the environmental context. The same mechanism, such as climatic limitation, resource availability or biotic interactions, may be determinant at some range edges, but secondary or even irrelevant at others (MacArthur, 1972; Paquette & Hargreaves, 2021; Radomski, 2026). This is particularly evident in complex systems such as the Andes, where high climatic variability, pronounced topographic heterogeneity and high species diversity generate a spatial mosaic of selective and ecological pressures that may differentially modulate distribution range limits.

In this context, a particularly interesting species is *Proctoporus lacertus* (Stejneger, 1913), a semifossorial lizard that inhabits the eastern slope of the Andes in southeastern Peru, from 13°04′S, where the mountain range begins to broaden, to 14°20′S, and eastwards to 72°17′W (Mamani et al., 2026). Altitudinally, it occurs between 2500 and 4400 m (Doan & Castoe, 2003; Goicoechea et al., 2013; Mamani et al., 2026). Across its distribution range, populations vary in body size. Forms associated with more arid environments, such as inter-Andean valleys and arid puna, tend to be smaller and slenderer, whereas populations from more humid environments, such as montane forests and humid puna, are larger and more robust (Mamani et al., 2026).

At its lower distributional limit, *P. lacertus* occurs adjacent to congeners such as *P. optimus*, *P. unsaacae*, *P. machupicchu* and *P. chasqui* (personal observation). In contrast, along much of its upper altitudinal limit, it is not altitudinally replaced by other gymnophthalmids (personal observation), except in the northernmost portion of its range, where it is latitudinally replaced by *Wilsonosaura josyi*, a species of similar body size. In the north-eastern sector, between the Vilcabamba and Vilcanota mountain ranges, *P. lacertus* exhibits reduced body size, which allows it to colonize the inter-Andean valley of the Vilcanota River, the Sacred Valley of the Incas (Mamani et al., 2026). It has been reported that, at the upper limit of this valley, on the right bank of the Vilcanota River, this population is replaced from 3590 m by *Proctoporus cf. kiziriani* (Doan & Castoe, 2003), a larger-bodied species comparable in size to *P. lacertus* populations from more humid areas (Fig. 1). This range edge is particularly informative because it combines an altitudinal transition, a shift in body size and spatial replacement between congeners, allowing us to evaluate whether the expansion of *P. lacertus* towards higher-elevation environments may be limited by interspecific competition.

Although the factors determining distribution ranges are multiple and context-dependent, the Sacred Valley of the Incas provides an opportunity to evaluate how interspecific competition may establish a distribution limit mediated by a trade-off between adaptation to arid environments and competitive performance. In this system, reduced body size in *P. lacertus* appears to favour its persistence in arid inter-Andean valley environments, but at the same time to decrease its competitive ability against a larger-bodied congener in contact zones. Thus, the expansion of *P. lacertus* towards the summit of the Cordillera de Vilcanota may be restricted not only by environmental conditions, but also by a competitive disadvantage relative to *P. cf. kiziriani*. In this scenario, larger body size may be crucial for dominance in access to limited resources, contributing to spatial replacement between both species and to the delimitation of the distribution range (Fig. 1). We propose the hypothesis that reduced body size in *P. lacertus*, associated with the colonization of arid environments such as the Sacred Valley of the Incas, entails a competitive cost against *P. cf. kiziriani*. This trade-off would favour the persistence of *P. lacertus* in lower-elevation arid environments, but limit its expansion into higher-elevation environments where it interacts with a larger-bodied congener, promoting spatial replacement and contributing to the establishment of the local upper limit of its distribution range.

Therefore, the objective of this study was to evaluate whether the competitive performance of *P. lacertus* against *P. cf. kiziriani* restricts the expansion of its distribution range, and whether this restriction depends on the body size of its populations. To do so, we conducted two common-garden experiments under conditions of limited food and nocturnal refuge: (i) *P. lacertus*-P vs. *P. cf. kiziriani* and (ii) *P. lacertus*-G vs. *P. cf. kiziriani*. We expected individuals of *P. lacertus*-P to show lower competitive

performance against *P. cf. kiziriani*, whereas individuals of *P. lacertus*-G should show equal or greater competitive ability.

3.3 MATERIALS Y METHODS

Specimens sampling and study area

We sampled individuals of *Proctoporus lacertus*-P, *P. lacertus*-G and *P. cf. kiziriani* from three localities in the department of Cusco, Peru. Individuals of *P. lacertus*-P were collected from the surroundings of the Pikillacta archaeological complex (13°12′34.57″S, 72°22′8.60″W; 2800 m a.s.l.), in the Piscacucho sector, on 20 November 2024. Individuals of *P. lacertus*-G were collected from Pacaymayu Alto (13°14′20.38″S, 72°29′30.25″W; 3860 m a.s.l.), along the Inca Trail within the Machu Picchu Historical Sanctuary, on 20 December 2024. Finally, individuals of *Proctoporus cf. kiziriani* were collected from the Colcas Peñas archaeological complex (13°10′19.59″S, 72°17′17.07″W; 3610 m a.s.l.) on 22 November 2024 (Fig. 2). In total, we obtained 36 specimens, corresponding to six adult males and six adult females from each locality.

Experimental design

We conducted common-garden experiments under natural light conditions in the city of Cusco, Peru (3300 m a.s.l.). For this purpose, we constructed glass enclosures measuring 80 × 30 × 30 cm, each subdivided into four 20 × 30 cm compartments, generating a total of 12 cohabitation units distributed across three experimental enclosures. Each compartment was conditioned with moss, used as refuge, and a small plastic container for water supply. To maintain substrate humidity, we sprayed water manually every night.

Competition for food resources

Before the start of each experiment, pairs cohabited for two weeks in the same space and were subsequently assigned randomly to the experimental units. During the first experiment, food consisted of brown cockroaches measuring 5–10 mm, whereas in the second experiment, individuals were fed crickets measuring 5–10 mm. Individuals were fed every 4 days; when feeding coincided with a weighing day, weighing was performed before food was provided. Water was available *ad-libitum* throughout the experiment.

We conducted the experiments in two phases. The first phase was carried out between 28 December 2024 and 5 February 2025. During this phase, we established mixed groups composed of one male and one female of *P. lacertus*-P, together with one male and one female of *P. cf. kiziriani*. The initial mass of each individual was recorded using an Ohaus Scout Pro balance with three-decimal precision, and individuals were subsequently weighed every 10 days, completing a total of 40 days of monitoring. The second phase of the experiment was structured in the same way, but using one male and one female of *P. lacertus*-G. This phase was carried out between 20 February and 31 March 2025, following the same experimental procedure.

Competition for refuge use

We conditioned the glass cages with moss to provide a nocturnal refuge, which was used consistently by all individuals during the two weeks prior to the competition trials. To evaluate the existence of interspecific competition for refuge access, we deliberately reduced the amount of moss. From day 20 onwards, we monitored refuge use until the end of the experiment. During this period, we recorded the species and sex of the individuals that remained outside the refuge at night and classified them as individuals displaced in the competition for access to the nocturnal refuge.

Additionally, we used two Ezviz C2C video surveillance cameras, which were rotated daily among the six experimental cages, obtaining continuous recordings for 24 hours per day. In the first experiment, recordings were conducted from 23 January until the end of the experiment, accumulating 35 days of video. All cages were recorded for six days, except cage 5, which was recorded for only five days, resulting in 35 days of video. In the second experiment, each of the six cages had six days of recording, resulting in 36 days of video. Videos were reviewed using VLC Media Player v3.0.21 (https://www.videolan.org/). Recordings from the first experiment were analysed between 07:00 and 14:00 h, and those from the second between 08:00 and 15:00 h, due to the later onset of activity by individuals. In total, we analysed 497 hours of video and recorded the frequency of offensive attacks, defensive attacks, intimidation behaviours and evaded attacks.

We classified agonistic interactions into five categories: (1) offensive attack, when an individual initiated an aggressive act through rapid approaches, charges or physical contact to displace or dominate the opponent; (2) defensive attack, when an individual responded aggressively to repel another individual invading its position; (3) attacks received, which included all aggressive acts directed towards an individual; (4) submissive behaviour, defined as avoidance or submissive behaviours through which an individual prevented contact with a rival; and (5) intimidating behaviour, when the presence or approach of an individual caused the opponent to retreat without physical contact. All these interactions were counted as independent events and, when repeated attacks occurred during an interaction, each attack was recorded separately.

Statistical analysis

We performed two-way analyses of variance (ANOVA) to evaluate whether there were significant differences in body mass between species and sexes at the beginning of each experiment.

To determine whether cohabitation affected body mass, we calculated mass variation (Δ = mass on day x – mass on day 0) on days 10, 20, 30 and 40. These day-specific comparisons corresponded to planned a priori contrasts, because the evaluated time points were established by the experimental design. We then analysed Δ as the response variable and, for each day and sex, compared Δ values between *Proctoporus lacertus* and *P. cf. kiziriani* using two-sample Welch's t-tests. Shapiro–Wilk normality tests indicated that data from the first and second experiments followed an approximately normal distribution; only a marginal deviation was observed in males of *P. lacertus* from the second experiment on day 20 ($W = 0.792$, $p = 0.049$). Nevertheless, Welch's t-tests were retained because they are robust to moderate deviations from normality.

To evaluate competition for refuge use, we applied generalized linear mixed models (GLMMs) with a binomial distribution. In both experiments, the response variable was nocturnal refuge use. Predictor variables included combinations of morphological traits: body mass, snout–vent length and tail length, and behavioural traits. The first set of models included species, body mass, sex, snout–vent length and tail length. In the second set, we incorporated agonistic variables. To make the effects of continuous predictors comparable and facilitate model convergence and interpretation, we standardized continuous variables using z-transformation, with mean = 0 and standard deviation = 1. Individual identity was included as a random effect to avoid pseudoreplication; cage and day were discarded because they did not explain additional variance.

We fitted four GLMMs: two comparing *P. lacertus*-P and *P. lacertus*-G with *P. cf. kiziriani*, and two additional models that included agonistic interactions. However, the models including agonistic variables showed convergence issues with the default optimizer; therefore, we excluded the morphological covariates, snout–vent length and tail length, and fitted the models by maximum likelihood using the *bobyqa* optimizer, increasing the maximum number of evaluations ($maxfun = 2 \times 10^6$) through *glmerControl*. To facilitate interpretation of species effects, we estimated marginal means and predicted probabilities on the response scale from the models using the *emmeans* package (Lenth & Piaskowski, 2025).

All analyses were performed using the *lme4* package (Bates et al., 2015) in R v.4.5.1 (R Core Team, 2025), and figures were generated using the *ggplot2* package (Wickham, 2016).

3.4 RESULTS

Variation in body mass

The comparison between *P. lacertus*-P and *P. cf. kiziriani* using a two-way ANOVA indicated a significant effect of species on body mass ($F_{1,20} = 131.99$, $p < 0.001$) and of sex ($F_{1,20} = 6.75$, $p = 0.017$). In addition, a significant interaction between species and sex was detected ($F_{1,20} = 8.18$, $p = 0.01$), indicating that sex differences varied between species. Accordingly, Tukey post-hoc comparisons revealed significant differences in the contrasts between sexes and species, except in *P. lacertus*-P, where no differences were detected between males and females (Table S1, Fig. 3).

In the comparison between *P. lacertus*-G and *P. cf. kiziriani*, the two-way ANOVA did not indicate a significant interaction between species and sex ($F_{1,20} = 2.44$, $p = 0.134$), nor a

main effect of species ($F_{1,20}$ = 1.05, p = 0.317). In contrast, a significant effect of sex on body mass was observed ($F_{1,20}$ = 7.51, p = 0.013), with differences between males and females being particularly evident in *P. cf. kiziriani* (Fig. 3). Tukey post-hoc comparisons are presented in Table S1.

Competition for food resources

In the first cohabitation experiment (*P. lacertus*-P vs. *P. cf. kiziriani*), mass variation (Δ) in females did not differ between species on any of the evaluated days ($p > 0.35$). In contrast, males of *P. cf. kiziriani* showed significantly higher Δ values than males of *P. lacertus*-P on days 10 (t = 2.60, df = 5.78, p = 0.042) and 20 (t = 2.85, df = 7.76, p = 0.022). On days 30 and 40, trends in the same direction were observed, although they were not significant (p = 0.060 and p = 0.067; Table 1, Fig. 4). In all cases, males of *P. cf. kiziriani* maintained higher body mass values relative to day zero.

In the second cohabitation experiment (*P. lacertus*-G vs. *P. cf. kiziriani*), mass variation (Δ) in females did not differ between species on any of the evaluated days ($p \geq 0.419$). In contrast, significant differences between species were detected in males across all measurements. *P. lacertus*-G showed higher Δ values than *P. cf. kiziriani* on days 10 (t = −2.47, df = 7.09, p = 0.042), 20 (t = −4.31, df = 7.76, $p < 0.003$), 30 (t = −3.86, df = 5.85, $p < 0.001$) and 40 (t = −2.84, df = 6.97, p = 0.025). In all cases, males of *P. lacertus* maintained consistently greater increases in body mass (Table 1, Fig. 4).

Competition for refuge use

In the first experiment on competition for refuge use, the binomial GLMM showed a positive and significant intercept (β = 4.349 ± 0.813; z = 5.352; $p < 0.001$), consistent with a high probability of refuge use under the reference conditions of the model (*P. cf. kiziriani*). Species had a marked effect: *P. lacertus*-P showed a significant reduction in

refuge use relative to *P. cf. kiziriani* (β = −4.732 ± 1.333; z = −3.549; $p < 0.001$), whereas sex did not show a significant effect (males: β = −0.370 ± 0.397; z = −0.931; p = 0.352). Among the morphological predictors, body mass was positively associated with refuge use (β = 1.906 ± 0.912; z = 2.090; p = 0.036), whereas snout–vent length showed a negative association (β = −2.925 ± 1.031; z = −2.838; p = 0.004) (Table 2). Accordingly, the estimated marginal means showed a higher predicted probability of refuge use by *P. cf. kiziriani* (0.985; 95% CI: 0.940–0.996) and a considerably lower probability in *P. lacertus*-P (0.362; 95% CI: 0.132–0.679).

In the second experiment on competition for refuge use, the intercept was not significant (β = 1.326 ± 1.223; z = 1.085; p = 0.278), suggesting high uncertainty in the probability of refuge use for the reference species under average conditions (*P. cf. kiziriani*). In this case, species showed the opposite pattern: *P. lacertus*-G showed a significant increase in refuge use relative to *P. cf. kiziriani* (β = 3.734 ± 1.425; z = 2.620; p = 0.009), whereas sex did not show a significant effect (males: β = −1.678 ± 1.249; z = −1.343; p = 0.179). Body mass showed a positive trend (β = 2.106 ± 1.184; z = 1.779; p = 0.075), but snout–vent length and tail length showed no detectable associations with refuge use (SVL: p = 0.363; tail: p = 0.753) (Table 2). Consistently, the estimated marginal means showed a higher predicted probability of refuge use by *P. lacertus*-G (0.986; 95% CI: 0.921–0.998) relative to *P. cf. kiziriani* (0.62; 95% CI: 0.248–0.889).

Competition for refuge use including agonistic interactions

For the first experiment on competition for refuge use incorporating agonistic interactions, the GLMM showed a positive and significant intercept (β = 3.508 ± 1.105; z = 3.174; p = 0.001), indicating a higher probability of refuge use for the reference species under average model conditions (*P. cf. kiziriani*). Species was also significant: *P. lacertus*-P showed a significant reduction in refuge use relative to *P. cf. kiziriani* (β =

−4.064 ± 1.953; $z = -2.081$; $p = 0.037$). Among the morphological predictors, body mass did not show a significant effect (β = −0.501 ± 0.967; $z = -0.518$; $p = 0.605$), whereas offensive attacks had a significant negative effect (β = −0.857 ± 0.406; $z = -2.109$; $p = 0.035$). Defensive attacks, intimidation behaviours and avoidance behaviours did not have significant effects (Table 2). Accordingly, the estimated marginal means showed a higher predicted probability of refuge use in *P. cf. kiziriani* (0.98; 95% CI: 0.825–0.998) relative to *P. lacertus*-P (0.43; 95% CI: 0.102–0.833).

For the second experiment, the GLMM showed a non-significant intercept (β = 0.896 ± 0.877; $z = 1.022$; $p = 0.307$), suggesting high uncertainty in the probability of refuge use for the reference species under average conditions (*P. cf. kiziriani*). Species had a significant effect: *P. lacertus*-G showed a higher probability of refuge use relative to *P. cf. kiziriani* (β = 4.484 ± 1.628; $z = 2.754$; $p = 0.006$). Among the morphological predictors, body mass was associated with a higher probability of refuge use (β = 1.823 ± 0.763; $z = 2.388$; $p < 0.017$). Agonistic predictors did not show significant effects (Table 3). The estimated marginal means showed a higher probability of refuge use in *P. lacertus*-G (0.98; 95% CI: 0.868–0.999) relative to *P. cf. kiziriani* (0.47; 95% CI: 0.191–0.776).

3.5. DISCUSSION

Our results provide experimental evidence that the outcome of competitive interactions between *Proctoporus lacertus* and *P. cf. kiziriani* depends strongly on the body size of *P. lacertus*. When individuals of *P. lacertus* corresponded to the small-bodied morphotype from the Sacred Valley of the Incas, *P. cf. kiziriani* showed greater competitive performance, reflected in larger increases in male body mass and a higher probability of nocturnal refuge use. In contrast, when *P. lacertus* corresponded to the large-bodied

morphotype from Pacaymayu, this pattern was reversed: males of *P. lacertus* increased their body mass more than those of *P. cf. kiziriani* and showed a higher probability of refuge use. Together, these results support our hypothesis that body size reduction associated with populations from arid environments may entail ecological costs by reducing competitive ability against *P. cf. kiziriani*. This pattern is consistent with the proposal that distribution limits can emerge from the interaction among local adaptation, evolutionary trade-offs and biotic interactions (Levin, 1970; Radomski, 2026; Willi & Van Buskirk, 2022).

Competition for food resources showed male-associated increases in body mass, whereas females did not show significant differences between species in either experiment. In the first experiment, the mass trajectory was consistent with a competitive advantage of male *P. cf. kiziriani*, reflected in more pronounced increases in body mass during the initial phase of the trial, on days 10 and 20. This suggests better access to resources under limiting conditions and higher probabilities of long-term persistence. In contrast, in the second experiment this pattern was reversed: males of *P. lacertus*-G showed significant increases in body mass during the experiment, whereas *P. cf. kiziriani* exhibited lower performance. However, we did not observe significant differences in mass variation among females. This could be because, although females exhibited greater agonistic activity (Fig. S1, Table S1), these interactions did not necessarily translate into effective resource monopolization. In addition, the energetic costs associated with agonism may have offset any advantage in access to resources. These observations are consistent with previous studies documenting the costs of aggression on feeding. For example, in *Podarcis erhardii*, the presence of conspecifics increases interruptions during feeding and delays the onset of consumption (Gavriilidi et al., 2025), whereas in *Sceloporus jarrovi*,

greater territorial defence increases energetic expenditure and requires compensation through greater access to food (Marler et al., 1995).

The results for nocturnal refuge use reinforce the interpretation of body size-dependent competition. In the first experiment, *P. lacertus*-P showed a significant reduction in refuge use relative to *P. cf. kiziriani*, with considerably lower predicted probabilities (0.36 vs. 0.98). In contrast, in the second experiment, this pattern was reversed: *P. lacertus*-G showed a higher increase in refuge use relative to *P. cf. kiziriani* (0.98 vs. 0.62). Given that refuge use is related to reduced physiological stress and predation risk in nature (Martín & López, 1999, 2010), losing access to refuge represents a direct cost to performance, with potential consequences for population survival. These results are consistent with the theory of asymmetric competition, in which body size determines dominance and small differences may confer an advantage in resource monopolization, ultimately leading to local exclusion of the subordinate species (Schwinning & Weiner, 1998). Although species of larger and smaller body size could partition the niche and coexist (Holt, 2017; Levin, 1970), in our study system we did not record populations of either *P. lacertus* or *P. cf. kiziriani* beyond their respective distribution limits. An additional possibility is that competitive asymmetry also varies with ontogeny, such that juveniles of *P. cf. kiziriani* may access more effectively the resources that could be used by *P. lacertus*-P and, consequently, reinforce the maintenance of the local range limit, as has been observed in other lizard species from Australia (Langkilde & Shine, 2007).

The literature reports that agonistic interactions can set range edges and slow or even prevent the colonization of novel environments, even when physical conditions appear to be permissive (Legault et al., 2020; Martin & Ghalambor, 2023; Senior et al., 2021). This effect is often particularly relevant in environments where phylogenetically close and similarly sized species coexist, because they tend to show high niche overlap and,

consequently, to compete for limiting resources such as food, refuges or breeding sites (Brown, 1971; Holt, 2017; Martin & Ghalambor, 2023). In these scenarios, the aggressor species may impose behavioural dominance, monopolize the resource and exclude the subordinate species, reducing its access to critical resources and limiting its performance. Such competitive asymmetries can translate into direct consequences for range expansion and range limits by restricting persistence or colonization in areas where the abiotic environment may be suitable.

However, our results did not show evidence that agonistic interactions substantively explain the exclusion of the subordinate species. The only exception was observed in the first experiment, where offensive attacks were negatively associated with the probability of refuge use, suggesting that individuals that attack more frequently have a lower tendency to occupy the refuge. This result indicates that dominance in access to refuges is not necessarily mediated by aggression per se, but may depend on other determinant characteristics, such as species identity and body mass. In this sense, our results are consistent with previous evidence suggesting that resource monopolization may be conditioned by individual traits and interspecific differences rather than by the intensity of aggression (Langkilde & Shine, 2007). Nevertheless, our results may also be influenced by design limitations, particularly the small sample size and experimental simplification, including six cages and a relatively short observation period.

From an evolutionary perspective, these results are consistent with a trade-off between adaptation to arid environments and competitive ability. Body size reduction in inter-Andean valley populations may favour persistence under conditions of lower water availability, greater thermal exposure and lower environmental productivity. However, this same phenotype may entail a disadvantage when individuals come into contact with larger-bodied congeners in higher-elevation environments with different abiotic

conditions. This type of trade-off has been proposed as an important mechanism shaping distribution limits, because traits that increase performance under abiotic stress may reduce competitive ability against resident species and better-adapted phenotypes (Hargreaves & Eckert, 2019; Willi & Van Buskirk, 2022). In addition, *P. lacertus*-P was competitively inferior to *P. cf. kiziriani* in access to food and refuge, whereas *P. lacertus*-G showed superior competitive performance. This result shows that body size can reverse the competitive hierarchy between both species. Consequently, if populations of *P. lacertus*-G were able to colonize the Cordillera de Vilcanota, they could reduce the competitive advantage of *P. cf. kiziriani* and favour range expansion of *P. lacertus*. However, this limit probably does not depend only on interspecific competition, but also on environmental filters such as physiological tolerances and climatic seasonality, or on possible synergistic effects among these factors (Gaston, 2009; Radomski, 2026; Sexton et al., 2009).

Additionally, during the release of *P. lacertus*-P individuals in the La Verónica Private Conservation Area, a relict montane forest adjacent to the Sacred Valley at 3070 m, we recorded an adult male of *P. cf. kiziriani* that was smaller than the individuals used in the experiment, but slightly more robust than co-occurring *P. lacertus*-P. This anecdotal record suggests that body size reduction in Andean gymnophthalmids may represent a more general pattern and that variation in body size may be associated with moisture gradients (Mamani et al., 2026). In this context, it is possible that *P. cf. kiziriani* may also reduce its body size in environments adjacent to arid valleys, which could compromise its performance in arid environments and favour its exclusion by *P. lacertus*-P. Together, this observation reinforces the hypothesis that body size reduction may be advantageous in arid environments, but entails costs during direct interactions with a resident species.

Finally, we recognize three relevant limitations in our study: (i) the treatments were conducted on different dates, with different prey types and the incidental presence of *Drosophila* in the environment, which could have introduced uncontrolled variation in food availability and, therefore, in changes in body mass; (ii) common-garden conditions, although useful for isolating mechanisms, simplify microhabitat heterogeneity and the natural dynamics of interactions; and (iii) inferences about interspecific competition require in situ validation through observations and experimental manipulation along the gradient. Even so, the consistency of the results between experiments and the phenotype-dependent directional shift strongly support our hypothesis.

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Ethical considerations

All procedures involving the capture, handling and temporary maintenance of individuals were conducted while minimizing stress and risk of injury. Fieldwork, collection and temporary holding of specimens for experimental purposes were carried out under the research permit issued by the National Forest and Wildlife Service of Peru (RD N° D000125-2024-MIDAGRI-SERFOR-DGGSPFFS-DGSPFS) and the Machu Picchu Historical Sanctuary (N° 009-2024-SERNANP-SHM/J). After the trials ended, individuals from the Sacred Valley of the Incas were released at their locality of origin, whereas individuals from the large-bodied population were euthanized to prevent potential hybridization and pathogen transmission among geographically differentiated populations.

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Figure 3.1 Distribution along the altitudinal gradient and variation in body size in *Proctoporus lacertus*. The arrow indicates the exclusion zone reported in the literature.

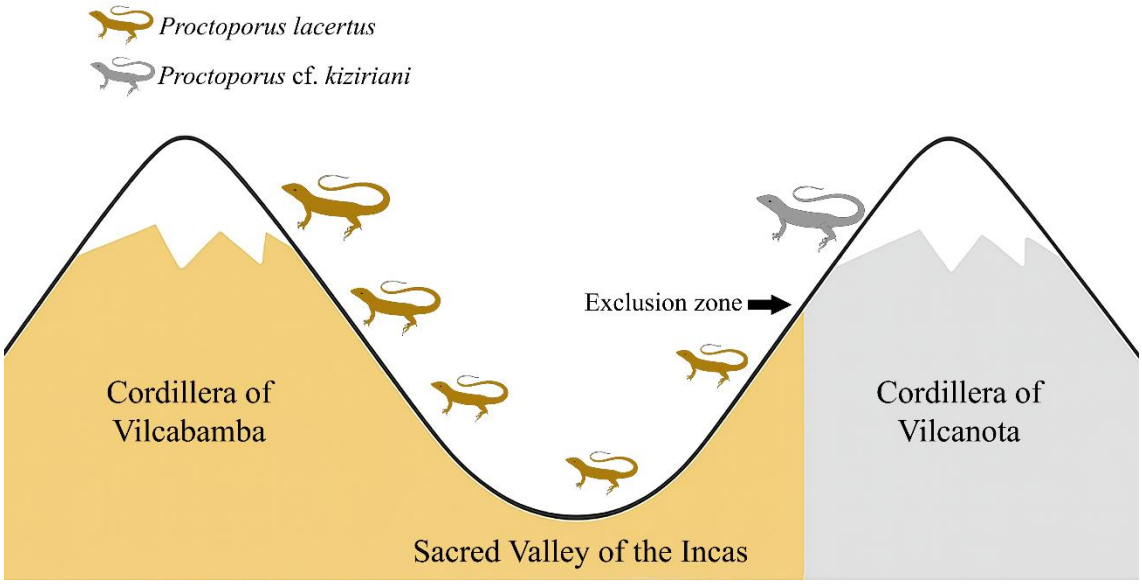


Figure 3.2 Location of the sampling localities used in the experiment. The light blue circle indicates *Proctoporus lacertus*-G from Pacaymayu; the white triangle indicates *P. lacertus*-P from Pikillacta; and the green pentagon indicates *P. cf. kiziriani* from Colcas Peñas.

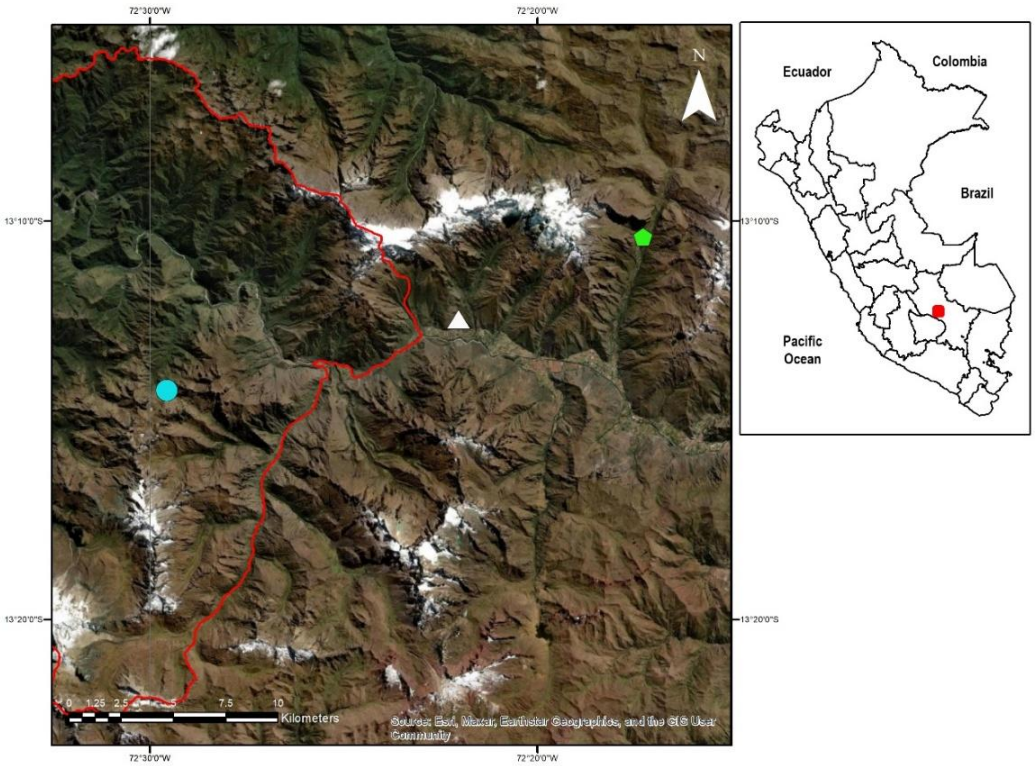


Figure 3.3 Boxplots of the initial body mass (g) of individuals used in the experiment. (A) *Proctoporus lacertus*-P vs. *P. cf. kiziriani*; (B) *P. lacertus*-G vs. *P. cf. kiziriani*. Asterisks indicate significant differences.

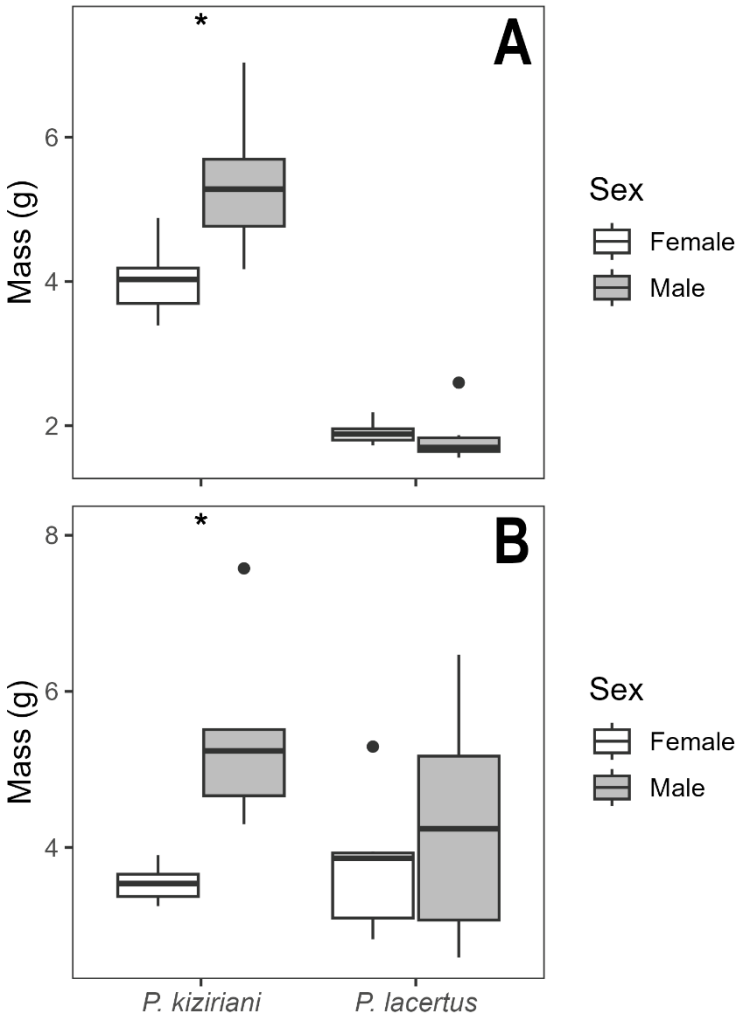


Figure 3.4 Variation in body mass of *Proctoporus lacertus* and *P. cf. kiziriani* over the 40 days of the experimental trials. (A) Experiment 1; (B) Experiment 2.

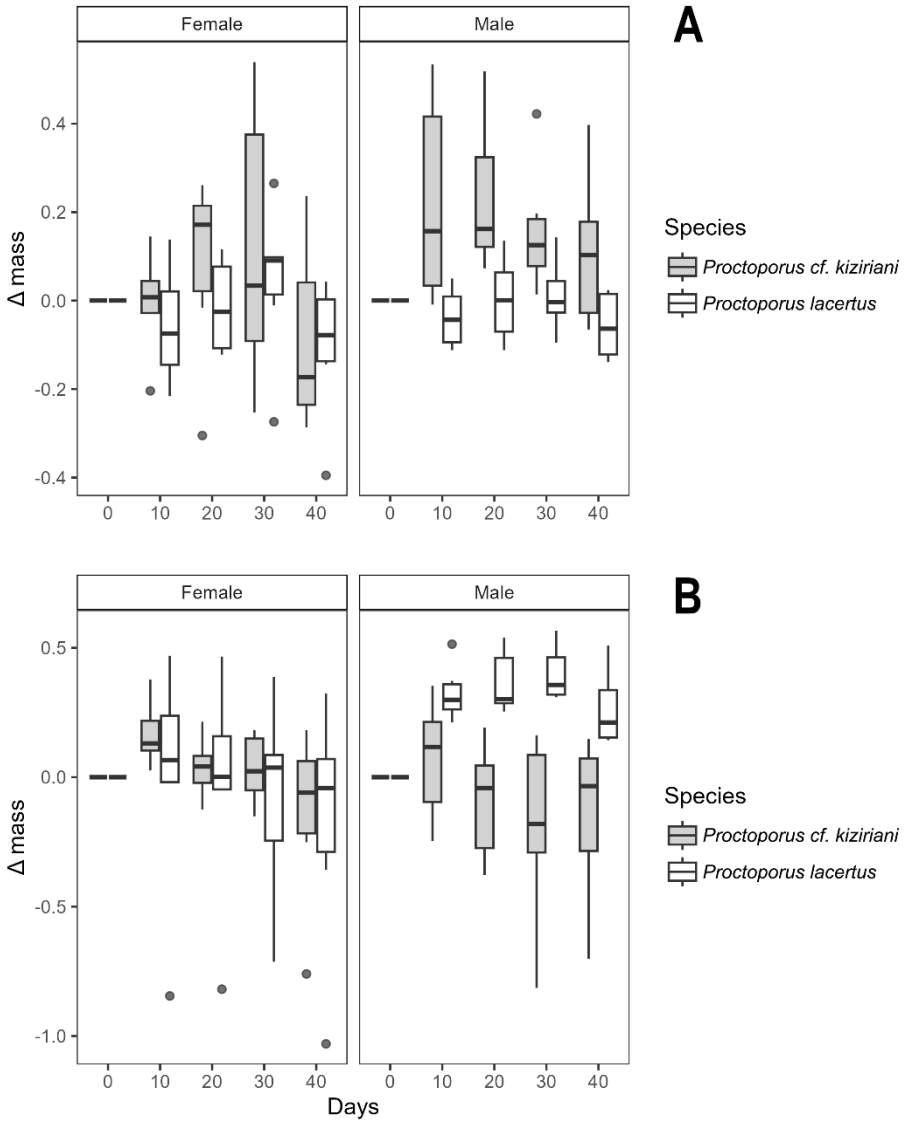


Table 3.1 Variation in body mass of *Proctoporus lacertus* and *P. cf. kiziriani* over the 40 days of the experimental trials. Bold values indicate significant differences.

Experiment	Day	Sex	t	gl	*p*	Δ *P. lacertus*	Δ *P.* cf. *kiziriani*	Diference (*kiz – lac*)
M1	10	Female	0.735	9.85	0.48	–0.0572	–0.00450	0.0527
	10	Male	**2.6**	5.78	**0.042**	–0.0388	0.222	0.261
	20	Female	0.99	7.37	0.353	–0.0135	0.0832	0.0967
	20	Male	**2.85**	7.76	**0.022**	0.00267	0.233	0.231
	30	Female	0.5	7.86	0.631	0.0435	0.118	0.0748
	30	Male	2.19	7.94	0.0598	0.011	0.159	0.148
	40	Female	0.145	9.31	0.887	–0.106	–0.0902	0.0157
	40	Male	2.17	6.92	0.0669	–0.057	0.112	0.169
M2	10	Female	0.871	5.75	0.419	0.0005	0.168	0.167
	10	Male	**–2.47**	7.09	**0.042**	0.325	0.0698	–0.256
	20	Female	0.392	5.72	0.709	–0.0338	0.0375	0.0713
	20	Male	**–4.31**	7.76	**<0.003**	0.365	–0.0913	–0.456
	30	Female	0.684	6.2	0.519	–0.0813	0.0323	0.114
	30	Male	**–3.86**	5.85	**<0.009**	0.399	–0.194	–0.593
	40	Female	0.133	9.03	0.897	–0.176	–0.144	0.0315
	40	Male	**–2.84**	6.97	**0.025**	0.264	–0.147	–0.412

Table 3.2 Results of the generalized linear mixed models (GLMMs) from the two experiments evaluating the effects of ecological variables on nocturnal refuge use.

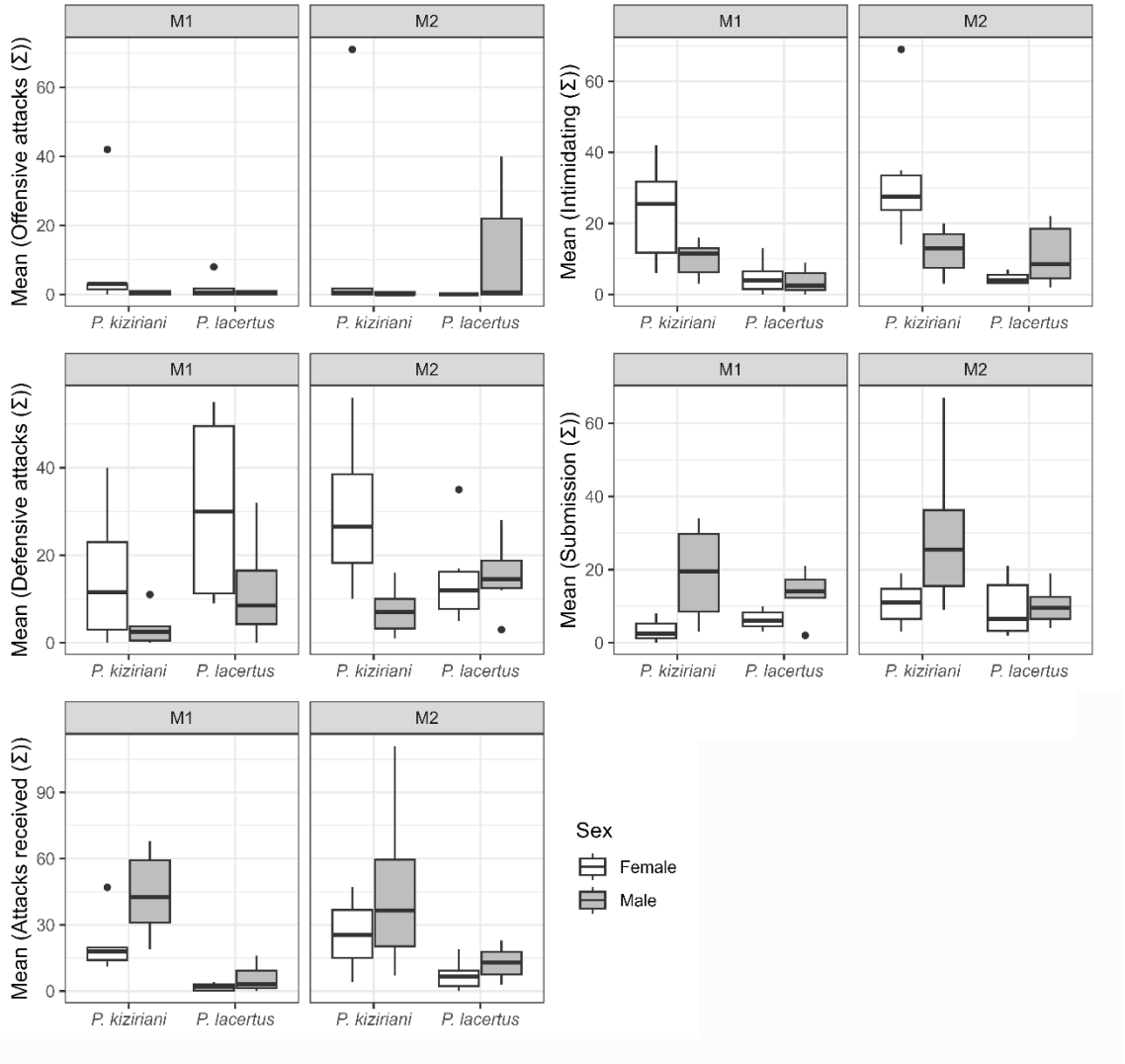
Experiment	Predictor	Estimate (β)	SE	z	*p*
M1	Intercept	4.349	0.813	5.352	**<0.001**
	Species (*P. lacertus*)	−4.732	1.333	−3.549	**<0.001**
	Sex (M)	−0.370	0.397	−0.931	0.352
	Masa	1.906	0.912	2.09	**0.036**
	SVL	−2.925	1.031	−2.838	**0.004**
	Tail	0.383	0.207	1.853	0.0638
M2	Intercept	1.3265	1.2226	1.085	0.278
	Species (*P. lacertus*)	**3.7338**	1.425	2.62	**<0.009**
	Sex (M)	–1.6779	1.2494	–1.343	0.179
	Mass	2.1063	1.1842	1.779	0.075
	SVL	–1.0582	1.1622	–0.911	0.363
	Tail	0.2126	0.6769	0.314	0.753

Table 3.3 Results of the generalized linear mixed models (GLMMs) from the two experiments evaluating the effects of ecological and agonistic variables on nocturnal refuge use.

Experiment	Predictor	Estimate (β)	Std. Error	z value	*p*-value
M1	Intercept	3.508	1.105	3.174	**0.001**
	Species (*P. lacertus*)	−4.064	1.953	−2.081	**0.037**
	Sex	0.539	0.786	0.685	0.493
	Mass	−0.501	0.967	−0.518	0.605
	Defensive attacks	−0.260	0.305	−0.852	0.394
	Offensive attacks	−0.857	0.406	−2.109	**0.035**
	Received attacks	−0.442	0.497	−0.889	0.374
	Intimidation	0.291	0.445	0.654	0.513
	Avoidance	0.503	0.391	1.289	0.197
M2	Intercept	0.896	0.877	1.022	0.307
	Species (*P. lacertus*)	4.484	1.628	2.754	**<0.006**
	Sex	−1.992	1.376	−1.448	0.148
	Mass	1.823	0.763	2.388	**<0.017**
	Defensive attacks	−0.394	0.358	−1.102	0.27
	Offensive attacks	−0.497	0.56	−0.886	0.375
	Received attacks	0.076	0.414	0.182	0.855
	Intimidation	−0.008	0.379	−0.020	0.984
	Avoidance	0.111	0.365	0.305	0.76

Supplementary material

Figura S3.1 Summary of agonistic interactions recorded per cage during the first (M1) and second experiment (M2).



CONCLUSIONES GENERALES

Los resultados de este estudio muestran las respuestas de una lagartija andina, *Proctoporus lacertus*, al filtro ecológico contrastante característico de la Cordillera de los Andes. La variación de rasgos morfológicos, en particular el tamaño corporal, y de rasgos térmicos se asocia con gradientes ecológicos de humedad climática y con el régimen térmico a lo largo del gradiente altitudinal. Además, encontramos evidencia de un *trade-off* en los ambientes áridos donde la reducción del tamaño corporal conlleva una disminución de la capacidad competitiva, lo que limita la colonización de otra montaña donde ocurre un conespecífico de mayor tamaño corporal y contribuye a delimitar el rango de distribución.

El tamaño corporal de *Proctoporus lacertus* varía significativamente a lo largo de gradientes ecológicos, con individuos de ambientes húmedos como bosques montanos y pajonales húmedos alcanzando mayores tamaños que aquellos de ambientes áridos. Asimismo, el dimorfismo sexual también dependió del ambiente, ya que los machos fueron significativamente más grandes que las hembras solo en ambientes húmedos, mientras que en ambientes áridos esta diferencia desapareció, lo que sugiere que la expresión del dimorfismo se ve favorecida cuando las restricciones energéticas e hídricas son menores y se atenúa bajo condiciones ecológicamente estresantes. Además, la disponibilidad hídrica fue el principal predictor del tamaño corporal, con individuos de mayor tamaño en ambientes más húmedos. En concordancia, la disponibilidad hídrica fue el principal predictor del tamaño corporal, probablemente porque los ambientes húmedos ofrecen microclimas más estables, mayor productividad y condiciones que permiten un crecimiento más prolongado. En contraste, los ambientes áridos, caracterizados por extremos térmicos, baja humedad, estacionalidad y menor disponibilidad de recursos,

impondrían restricciones al crecimiento y favorecerían tamaños corporales menores y una madurez más temprana.

Asimismo, se evaluó la ecología térmica de *Proctoporus lacertus* a lo largo de ~1000 m de gradiente altitudinal. Los resultados muestran una asimetría frío–calor, con una variación más marcada de CT_{min} respecto a CT_{max}, coherente con que la exposición al frío nocturno es más inevitable que la exposición al calor diurno, que puede amortiguarse mediante el uso de refugios. En la localidad más elevada, T_e alcanzó mínimos bajo 0 °C en todos los microhábitats y máximos diurnos extremos en hábitats expuestos; Además, presentó un rango de tolerancia (R_{tol}) mayor que las otras localidades, explicado principalmente por una reducción de CT_{min}. VT_{min} también fue menor en Pacaymayu, lo que es consistente con la capacidad de mantener la actividad a temperaturas más bajas. En contraste, T_{sel}, VT_{max} y R_{vol} se mantuvieron conservados entre localidades, un patrón compatible con la amortiguación conductual. A pesar de T_e extremadamente variable, T_b se mantuvo relativamente acotada y la termorregulación fue distinta de cero, con mayor precisión y efectividad en altura pese a una menor calidad térmica del hábitat, lo que sugiere que la termorregulación es necesaria. Además, al traducir T_e en horas potenciales de supervivencia y actividad, se observó un fuerte desacople entre ambas y una dependencia marcada del microhábitat, con máximos de actividad en refugios y mínimos en microhábitats expuestos, lo que vincula de forma mecanicista el uso de refugios y hábitos semifosoriales. Estos resultados sugieren que una amenaza clave no es solo el calentamiento global, sino la pérdida de cobertura vegetal y de complejidad del hábitat que podría erosionar el mosaico térmico y reducir el tiempo utilizable para actividades relacionadas al *fitness*.

Finalmente, este estudio encontró que los límites del rango de distribución de *P. lacertus* en la parte nororiental puede explicarse por la competencia interespecífica con un

congénere cercano. En particular, la reducción del tamaño corporal asociada a condiciones áridas del valle se alinea con un *trade-off*, donde este fenotipo parece ser ventajoso localmente para persistir bajo estrés, pero implica costos bajo competencia directa, porque disminuye la capacidad de monopolizar recursos clave como alimento y refugios frente a *P.* gr. *kiziriani*. Además, el desenlace competitivo fue dependiente del fenotipo poblacional, las poblaciones de *P. lacertus* de menor tamaño fueron subordinadas bajo recursos limitados, mientras que poblaciones de mayor tamaño revirtieron la asimetría y mostraron superioridad competitiva. En conjunto, estos hallazgos apoyan que el borde altitudinal local corresponde a un límite de nicho realizado impuesto por interacciones bióticas, cuyo resultado puede variar en función de la variación intraespecífica del tamaño corporal.

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