# ARTICLE

# Chromatic variability and sexual dimorphism in the rocky lizard *Phymaturus verdugo*

V. Corbalán, N. Vicenzi, D.L. Moreno Azócar, and S. Literas

**Abstract:** The genus *Phymaturus* comprises lizards that inhabit cold environments at high altitudes or latitudes. *Phymaturus verdugo* Cei and Videla, 2003 is characterized by cephalic melanism, which is interpreted as a character present only in males and associated with sexual dimorphism. Using spectrophotometry and photography, we demonstrate that this species has high chromatic variability and that melanism is also present in females. By comparing colour variables on 15 patches of the body, we find significant differences between sexes in all patches, indicating strong sexual dichromatism for the entire body, not only the cephalic region. Body temperature had no effect on skin reflectance or melanism, which indicates that thermoregulation early in the day is not mediated by colour changes. Contrary to our predictions, we found no evidence of better body condition in darker individuals. Our study breaks some paradigms about the genus *Phymaturus* and suggests that human perception of colour, as well as working with small samples, can lead to misidentification or erroneous descriptions of species. We believe that future studies should explore heating rates and other functions of melanism such as crypsis, sexual selection, immune response, and UV protection to understand chromatic variability within this genus.

*Key words:* body condition, colouration, sexual dichromatism, skin reflectance, melanism, *Phymaturus verdugo*, three-coloured dragon.

**Résumé :** Le genre *Phymaturus* comprend des lézards qui habitent des milieux froids à hautes altitudes ou latitudes. *Phymaturus verdugo* Cei et Videla, 2003 est caractérisé par un mélanisme céphalique qui est interprété comme étant un caractère présent seulement chez les mâles et associé au dimorphisme sexuel. En utilisant la spectrophotométrie et la photographie, nous démontrons que cette espèce présente une forte variabilité chromatique et qu'un mélanisme est également présent chez les femelles. En comparant des variables chromatiques sur 15 parcelles du corps, nous notons des différences significatives entre les sexes dans toutes les parcelles, ce qui indique un fort dichroïsme sexuel pour tout le corps et non la seule région céphalique. La température du corps n'a pas d'effet sur la réflectance de la peau ou le mélanisme, ce qui indique que la thermorégulation tôt durant la journée n'est pas médiée par des changements de couleur. Contrairement à nos prédictions, nous ne notons aucun indice d'un meilleur embonpoint chez les individus plus foncés. L'étude invalide certains paradigmes concernant le genre *Phymaturus* et donne à penser que la perception de la couleur par les humains et le fait de travailler avec de petits échantillons peuvent mener à des identifications ou descriptions d'espèces erronées. Nous croyons que les études futures devraient se pencher sur les taux de réchauffement et d'autres fonctions du mélanisme comme le crypsis, la sélection sexuelle, la réaction immunitaire ou la protection contre les rayons UV pour comprendre la variabilité chromatique au sein de ce genre. [Traduit par la Rédaction]

*Mots-clés :* embonpoint, coloration, dichroïsme sexuel, réflectance de la peau, mélanisme, *Phymaturus verdugo*, dragon tricolore.

## Introduction

Colour variation in animals is generally interpreted as an adaptation to local environmental conditions and selective agents such as substrate colour or environmental temperatures (Stuart-Fox et al. 2004; Farallo and Forstner 2012; Reguera et al. 2014). However, in some species, colour may also vary with breeding season (McGuire 1996; Carretero 2002; Macedonia et al. 2002; Germano and Williams 2007), social status or signalling (Baird et al. 1997; Macedonia et al. 2002; Stuart-Fox and Mousalli 2008), light conditions (Vroonen et al. 2012), or body temperature (Walton and Bennett 1993; Rosenblum 2005).

Melanism or dark colouration is a clear type of colour variation. In reptiles, melanism is caused by melanophores that produce melanin in the form of eumelanin, which ranges from dark brown to black pigments (Bagnara and Hadley 1973; Morrison et al. 1995). Changes in dorsal colour are due to variable production and dispersion of these granules (Hadley 1997). It was proposed that the pleiotropic effects of the melanocortin system, which controls the synthesis of melanin, might account for the covariance between melanin-based colouration and other phenotypic traits (Ducrest et al. 2008). Consequently, the melanocortin system has been a focus of ecological and evolutionary adaptation studies (Escudero et al. 2016) such as the relationship between melanin-based colouration and sexual behaviour. Melanocortins enhance female fertility and sexual receptivity, as well as male sexual motivation,

**V. Corbalán and N. Vicenzi.** Instituto Argentino de Investigaciones de las Zonas Áridas (IDIZA–CONICET), Centro Científico y Tecnológico (CCT Mendoza–CONICET), Avenida Ruiz Leal s/n, CP 5500, Mendoza, Argentina.
**D.L. Moreno Azócar.** Laboratorio de Fotobiología, Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), CONICET–UNCOMA, Quintral 1250, Bariloche, 8400, Río Negro, Argentina.
**S. Literas.** Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza. Almirante Brown 500, M5528AHB Chacras de Coria-Luján de Cuyo, Mendoza.
**Corresponding author:** Valeria Corbalán (email: corbalan@mendoza-conicet.gob.ar).

performance, and aggressiveness (Ducrest et al. 2008). Melanin also reduces apoptosis (Chai et al. 2006), oxidative stress, and DNA damage induced by UV radiation on the skin (Böhm et al. 2005). It may also have an important immune function by reducing allergic and systemic inflammation and septic shock and improving recovery after ischaemia (Getting 2006). Melanin production is complex and energetically expensive, thus the amount of melanin could track individual health, genetic quality, and condition of an individual (Jawor and Breitwisch 2003). Given the proposed advantages of melanin, darker animals should be healthier than lighter ones.

Darker ectotherms could have a selective advantage in cold regions because they can absorb more solar radiation (Porter and Gates 1969). Melanistic animals heat faster, more quickly reach optimal body temperatures, and have more time for developing other activities (Clusella-Trullas et al. 2009; Vroonen et al. 2012). In the same way, rapid colour change in reptiles has often been associated with thermoregulation (Walton and Bennett 1993). Several diurnal species are dark when they emerge to bask and blanch as their body temperature rises (Norris 1967; Walton and Bennett 1993; Sherbrooke et al. 1994; Rosenblum 2005). Although dark colour in cold animals allows them to heat faster, pale colouration at higher body temperatures may reduce the risk of overheating by decelerating heating rates (Vroonen et al. 2012).

For Argentine lizards, the role of melanism on body condition and thermoregulation has only been studied for species of the genus *Liolaemus* Wiegmann, 1834. Escudero et al. (2016) tested the relationship between ventral melanism and body condition in the Fitzinger's Tree Iguana (*Liolaemus fitzingerii* (A.M.C. Duméril and Bibron, 1837)) group, but no effect could be demonstrated. Moreno Azócar et al. (2016) tested the effects of morphology and colour on heating and cooling rates with live and euthanized lizards from the clade *Liolaemus goetschi* L. Müller and Hellmich, 1938. They did not find a clear pattern because the influence of dorsal melanism on heating by radiation was masked by body mass in live animals.

In this paper, we present the first study on melanism in lizards of the genus *Phymaturus* Gravenhorst, 1838, the sister group of the genus *Liolaemus*. Species of the genus *Phymaturus* are saxicolous, herbivorous, and viviparous; they inhabit the Chilean and Argentine Andes, as well as extra Andean ecosystems such as Payunia and the Patagonian steppe. The literature reports that although southern *Phymaturus* species (belonging to the Patagonia Mountain Lizard (*Phymaturus patagonicus* Koslowsky, 1898) group) may include species or morphs with complete melanism, in northern species (that belong to the High Mountain Lizard (*Phymaturus palluma* (Molina, 1782)) group), melanism is only present in the cephalic region (Lobo and Nenda 2015). Lobo and Nenda (2015) affirm that in the *palluma* group, unlike the *patagonicus* group, melanism is determined by sexual dimorphism and only present in males.

The rocky lizard *Phymaturus verdugo* Cei and Videla, 2003 is known locally as the three-coloured dragon (dragoncito de tres colores). It is the most melanic species in the *palluma* group and was named for the deep black cephalic hood of males. The original description remarks that males have minor differences in the extent of black pigmentation on the head, neck, shoulder, forelimb, and chest; larger and darker areas depend on age and body size. Females have smaller dark areas such as bilateral dark bands on the head, lateral nuchal folds, and shoulders; it almost vestigial in some individuals. However, we have recently observed great variability in *P. verdugo* colouration both within and among populations, which led us to the goals of this study: (*i*) to test for correlations between sex, body size, and colour variability, which was measured by spectrophotometry and photography, and (*ii*) to assess the relationship between melanism, body condition, and thermoregulation. We expect better body conditions in darker individuals, as well as lower brightness at lower body temperatures.

## Materials and methods

*Phymaturus verdugo* inhabits rocky promontories across the Andes highlands in the southwest part of the Malargüe department in Mendoza, Argentina. Throughout its restricted distribution range, this endemic species shows great chromatic variability (Figs. 1A–1I) within and between populations.

The region's weather is attenuated periglacial (Capitanelli 1999). Temperatures range from −6.2 °C (August) to 21.5 °C (January) (Ortíz Maldonado 2001). The area is influenced by westerlies and the South Pacific cyclone, with heavy snowfalls prevailing in winter (Trombotto Liaudat et al. 2014) when lizards are inactive.

During January 2016 and January 2017, we collected by noose a total of 108 individuals (54 each year) from different rocky promontories along highways 145 and 226. The northernmost collection site was located at 35°26′23.99″S and 70°15′00″W at 1894 m above sea level and the southernmost site was located at 35°57′36″S and 70°13′48″W at 1987 m above sea level.

To identify individuals, we numbered them with permanent ink on their bellies. At the moment of capture, body temperature ($T_b$) was recorded using TES TP-K thermocouples connected to a TES 1312A digital thermometer (range: −50 to 1300 °C; resolution: 0.1/1 °C). Coordinates of the capture site were determined with a GPS (Garmin Etrex 30) and marked with a flag to later release the individuals at the same location. All procedures were performed with permission of the provincial authority (Dirección de Recursos Naturales Renovables, Res. Nos. 989 and 1570) and comply with Argentinian National Law 14346 on animal care. Captured individuals were carried in individual cloth bags to a laboratory where we recorded sex (based on the presence or absence of precloacal pores, which are evident only in males), mass (with a digital balance Precision TH200, with a capacity of 200 g and an accuracy of 0.1 g), and snout–vent length (SVL) (with a Stronger digital caliper, 0.01 mm accuracy). Females with a SVL greater than 93 mm were considered adults (based on the body size of the smallest gravid females). Males with a SVL greater than 100 mm were considered adults (based on the SVL of the smallest individual with orange precloacal pores). Some juveniles presented black precloacal pores and were considered males.

### Colouration

We used spectrophotometry and photography to measure colouration. Spectrophotometry provides objective, quantitative data for specific body patches, whereas photography made it possible to quantify the distribution and the proportion of melanic skin.

Reflectance measurements were taken at two body temperatures in 2016. To do this, 49 adult lizards (28 males and 21 females) were left in a dark room at 20 °C overnight and measured early in the morning. Lizards were then warmed up by solar radiation or UV lamps and measured again once body temperatures reached 32 ± 1 °C. In 2017, reflectance was recorded only at 32 ± 1 °C in 54 adult individuals (25 males and 29 females). No individual was measured twice because they were captured in different localities.

Visible spectral reflectance (400–700 nm) was measured at 0.42 nm intervals. Reflectance was measured on 15 patches of the body (6 dorsal, 1 dorsolateral, 3 lateral, and 5 ventral) using a JAZ EL200-XR1 spectrophotometer (Ocean Optics, Inc.). A bifurcated optical fibre cable was used with a PX-2 pulsed xenon light source; data were collected at 45° to the body surface. The tip of the probe was covered by a black rubber tube to block ambient light but maintain 5 mm between the probe and the skin. The end of the tube was cut at 45°, resulting in an elliptical patch of 10 mm². Reflectance measures were obtained by setting up 10 scans to average and Boxcar width 5 using Ocean View version 1.6.5 software (Ocean Optics Inc.). We calibrated using a WS-1 diffuse reflectance standard; white and black measures were taken every 20 min. Dorsal patches were the head, nape, interscapular region,

**Fig. 1.** Chromatic variability in the rocky lizard *Phymaturus verdugo*: (A) highly melanic male with dorsal reticulations; (B) highly melanic male without dorsal reticulations; (C and D) slightly melanic males; (E) highly melanic female; (F) slightly melanic female with small reticulations; (G) reddish female; (H) brownish female with highly cephalic melanism; (I) highly melanic juvenile. Photos B, G, and H were taken outside the study sites.
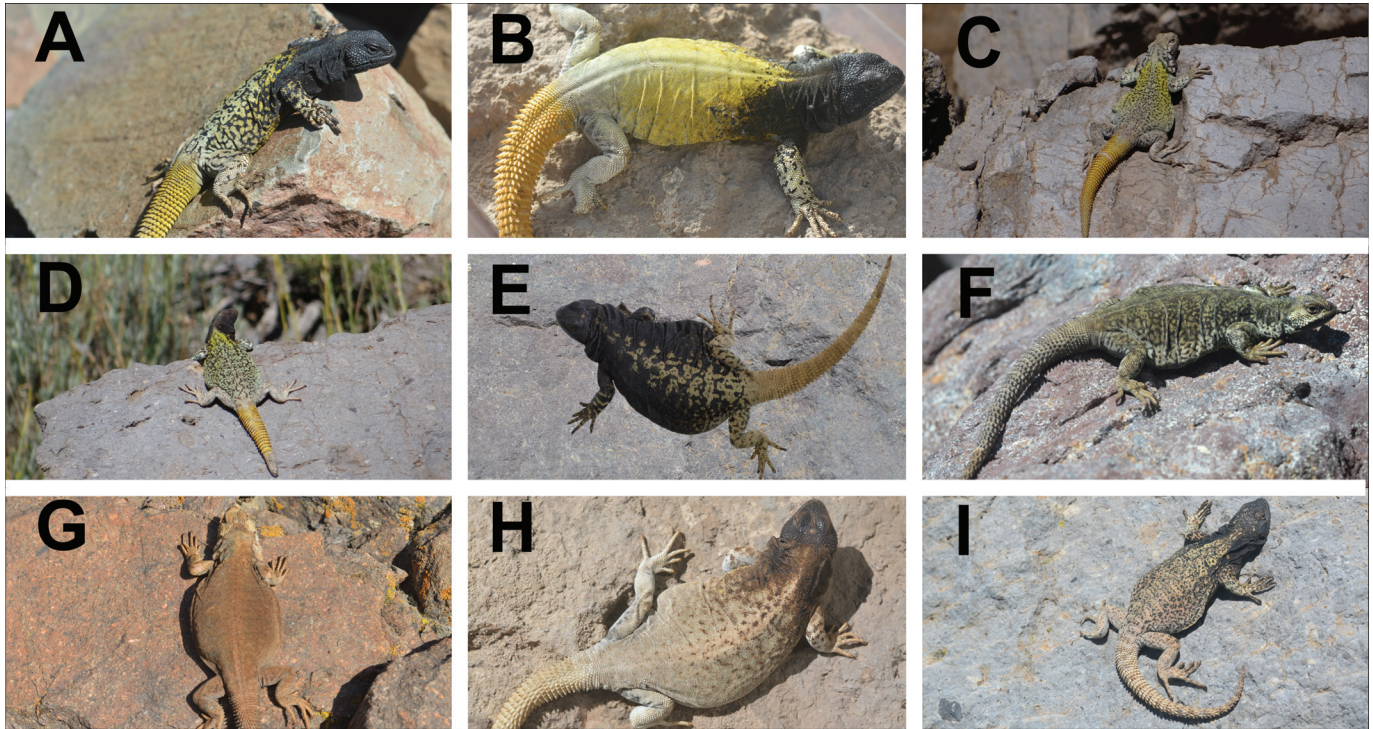
middle dorsum, posterior region (between the hind legs), and tail. The dorsolateral measure was the flank. Lateral measures were the cheek, shoulder, and elbow. Ventral measures were the chin, throat, chest, belly, and abdomen (Figs. 2A, 2B).

Spectral curves were analysed separately for each patch. We quantified colour using the segment classification method proposed by Endler (1990). Segments correspond to the blue (400–474 nm), green (475–549 nm), yellow (550–624 nm), and red (625–700 nm) parts of the visible spectrum. The shape and height of reflectance spectra are described by three variables: brightness (total area under the curve), chroma (or saturation, slope of the curve as it approaches peak reflectance), and hue (the wavelength with the highest reflectance). These variables were calculated following Smith's (2014) protocol, which provided improved formulae for hue. Typically, melanic individuals have lower skin reflectance than nonmelanic individuals (Clusella-Trullas et al. 2009; Geen and Johnston 2014). Dark individuals (with lower skin reflectance) absorb more solar radiation than lighter ones (with higher reflectance) (Clusella-Trullas et al. 2008); thus, the variable of major interest to evaluate thermoregulatory function of colouration is brightness because the area under the curve determines the darkness of animals. Chroma is a measure of the "purity" or "saturation" of a colour and is a function of how rapidly intensity changes with wavelength (Endler 1990). It varies in relation to the contrast in relative brightness in segments of the spectrum (the strongest reflectance differences between yellow and blue parts of the visible spectrum or between red and green parts of the visible spectrum, the highest chroma values). Hue values mark points in a colour space; they are lower for red parts of the spectrum and increase progressively for yellow, green, and blue parts of the spectrum (Smith 2014). Chroma and hue may be informative about nonmelanistic skin like in the dorsum, posterior region, or ventral patches. Differences between sexes in any of these values may indicate sexual dichromatism.

To assess sexual dichromatism, we performed a multivariate analysis of variance (MANOVA) of brightness, chroma, and hue for each of the 15 skin patches. We performed Hotelling's $t^2$ test with Bonferroni correction to control for type I errors. Analyses were done with the program InfoStat (2009).

We used photography to quantify the proportion of melanic skin at two body temperatures (20 ± 1 and 32 ± 1 °C), following the procedure described above. Photographs were taken for all individuals captured in 2016. We used a Nikon D3200 digital camera and Nikkor 50 mm f/1.86 lens, with the diaphragm set to f/16, ISO sensitivity to 800, and automatic shutter speed. The camera was placed on a tripod to take orthogonal photos with a constant distance between the lens and the lizard and to minimize shadows. Lizards were gently placed on a white background into a box. We took dorsal and ventral photographs, but only dorsal views were analysed because arms obstructed the abdomen in some ventral photographs. Images were processed with the program ImageJ2 (Schindelin et al. 2015). We quantified the total dorsal area using polygon selection and used the freehand tool to remove the forelimbs, hindlimbs, and tail. The background was eliminated using the magic wand (tracing) tool. The colour photograph was transformed to an 8-bit grey-scale image with colour intensity values from black (0) to white (255). We classed pixels with values of 0–100 as dark. We used the areas of dark and nondark pixels to calculate the proportion of melanism for each individual.

SVL is an indirect measure of age because lizards grow continuously and *Phymaturus* species in the *palluma* group can live 18–20 years (Boretto et al. 2015, 2018). To evaluate whether melanism is a character related with body size, we performed a logistic regression between SVL and the proportion of melanism for juveniles and adults. Sex was included as a factor and the interaction between this variable and the proportion of melanism was assessed. As data showed overdispersion, a quasibinomial distribu-

**Fig. 2.** Body patches of the rocky lizard *Phymaturus verdugo* where reflectance was measured. (A) Dorsal and lateral patches: 1, head; 2, nape; 3, interscapular region; 4, dorsum; 5, posterior region; 6, tail; 7, cheek; 8, shoulder; 9, elbow; 10, flank. (B) Ventral patches: 11, chin; 12, throat; 13, chest; 14, belly; 15, abdomen.
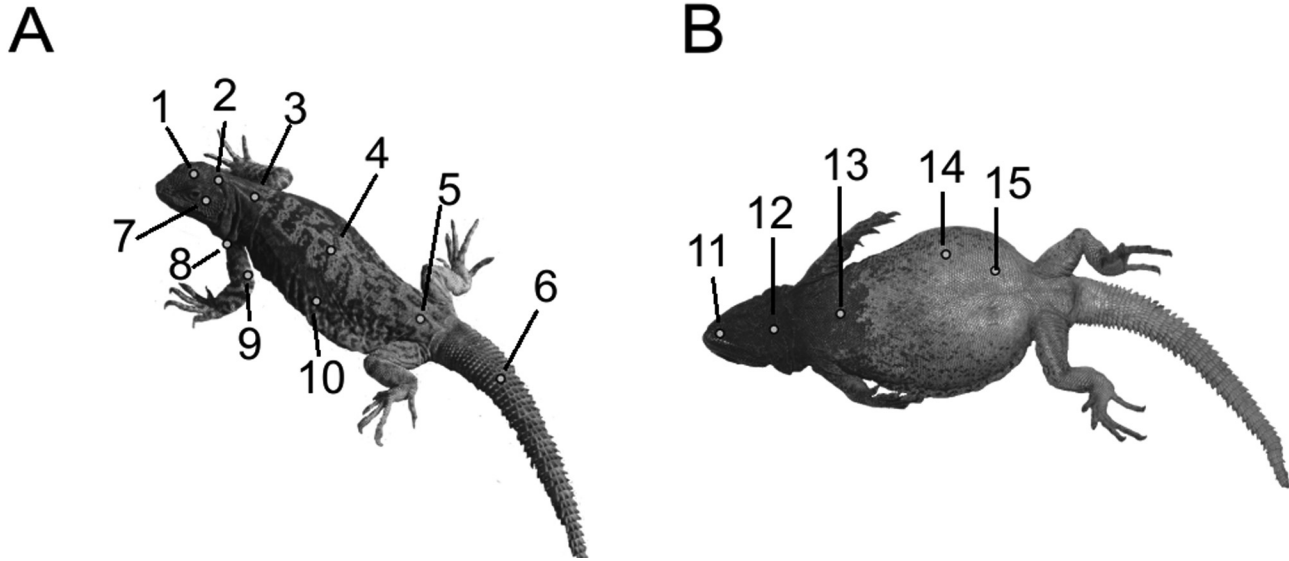


tion was used instead of a binomial distribution. The goodness of fit was evaluated by the Hosmer–Lemeshow test using the ResourceSelection package (Lele et al. 2017). Analyses were performed using R (R Core Team 2015).

**Melanism and body condition**

To infer body condition, we used the scaled mass index (SMI) proposed by Peig and Green (2009). Because SMI is assumed to influence an animal's health and fitness, it represents a tool to assess the relationship between melanism and health in ectotherms (Peig and Green 2009). It is calculated as $\text{SMI} = M_i \cdot (L_0/L_i) \cdot b^{\text{SMA}}$, where $M_i$ and $L_i$ are the body mass and linear body measures of individual $i$, respectively; $L_0$ is the arithmetic mean value for the study population; and $b^{\text{SMA}}$ is the slope of a standardized major axis (SMA) regression of ln mass on ln SVL for the population (Peig and Green 2009, 2010). The $b^{\text{SMA}}$ exponent was calculated using the lmodel2 package (Legendre 2015) in R (R Core Team 2015). SMI was calculated for adults captured in 2016 ($n = 51$), but pregnant females were excluded. We used a linear model that used body condition as the response variable and proportion of melanism and sex as explanatory variables. The interaction between these variables was assessed and only significant variables and interactions were kept in the final analysis. Proportion of melanism was transformed by taking the arcsine of the square root. Analyses were performed using R (R Core Team 2015).

**Thermoregulation**

To test whether darkness influences thermoregulation, we compared colouration and proportion of melanism at two body temperatures. If lizards are darker when colder, then we can presume, as was suggested for other species, that darker skin helps lizards gain energy and heat faster, whereas brighter skin at higher body temperatures reduces energy gain, diminishing the risk of overheating (Vroonen et al. 2012). The lack of colour change with temperature, however, does not mean that colour does not affect heat gain. For this analysis, we compared the ratio of dorsal melanism at different temperatures with paired $t$ tests. This test was also used to compare the mean brightness values of dorsal and lateral patches for each individual at different temperatures. We discarded ventral data because these patches are not influenced by solar radiation and are unrelated to thermoregulation. As no differences were found (see the Results), we repeated the

analyses for each patch and colour variable (brightness, chroma, and hue).

## Results

**Colouration and its relationship to sex and size**

We found high variability in colouration between and within the sexes (Table 1, Fig. 3). MANOVA tests revealed that all patches showed significant colour differences between the sexes (Table 1). Within each sex, both standard deviations and coefficients of variation (CV) were high in most skin patches, especially in brightness. Lateral and anteroventral patches showed the highest variability values. Hue in male dorsal patches was significantly higher than in females, as well as the majority of the lateral patches and abdomen (Table 1, Fig. 3). That is, although reflectance curves of females peak in the red part of the spectrum, males show peaks at shorter wavelengths, including the yellow and red parts of the spectrum (Fig. 3).

The proportion of melanism was highly variable among individuals in both sexes, with ranges of 40.37%–87.75% in males and 11.61%–90.49% in females. The logistic regression showed no relationship between SVL and proportion of melanism, nor interaction between sex and SVL ($t = -0.28$, $p = 0.77$, $n = 54$). The only significant variable was sex, after removing the interaction from the model, which indicated that males have higher proportion of melanism than females ($t = 2.21$, $p = 0.03$, $n = 54$).

**Melanism, body condition, and thermoregulation**

Body condition index was not influenced by percentage of melanism or the interaction between percentage of melanism and sex. The final model only includes sex ($F_{[1,49]} = 17.09$, $p = 0.0001$, adjusted $R^2 = 0.24$, $n = 49$), indicating that males have higher body condition indices than females.

Proportion of melanism did not vary with body temperature (paired $t$ test: $t = -1.13$, $p = 0.26$, $n = 43$). Reflectance curves confirmed these results, as mean brightness did not differ (paired $t$ test: $t = -0.05$, $p = 0.957$, $n = 49$). When patches were analysed separately, we only found significant differences for tail brightness (paired $t$ test: $t = 2.22$, $p = 0.03$, $n = 49$) (Fig. 4). However, we found significant differences in chroma for the head (paired $t$ test: $t = 2.16$, $p = 0.03$, $n = 49$) and tail (paired $t$ test: $t = 3.03$, $p = 0.004$, $n = 49$), and in hue for the nape (paired $t$ test: $t = 2.32$, $p = 0.02$, $n = 49$),

**Table 1.** Results of MANOVA performed for each measured patch to evaluate sexual dichromatism in the rocky lizard *Phymaturus verdugo*.

| Body part | Colour variable | Female | Male | CV (%) Female | CV (%) Male | F | df | p |
|---|---|---|---|---|---|---|---|---|
| Head | Brightness | 57.42±30.36 | 46.06±24.73 | 52.87 | 53.69 | 12.85 | 3, 99 | <0.0001 |
| | Chroma | 0.2±0.06 | 0.14±0.05 | 28.27 | 37.40 | | | |
| | Hue | 45.23±10.17 | 46.94±15.49 | 22.47 | 33.01 | | | |
| Nape | Brightness | 95.95±41.02 | 76.57±45.69 | 42.77 | 59.67 | 5.79 | 3, 99 | 0.0011 |
| | Chroma | 0.17±0.04 | 0.14±0.04 | 23.27 | 29.32 | | | |
| | Hue | 55.96±14.65 | 64.96±21.29 | 26.18 | 32.77 | | | |
| Interscapular | Brightness | 63.97±23.36 | 71.37±41.69 | 36.52 | 58.42 | 17.31 | 3, 99 | <0.0001 |
| | Chroma | 0.22±0.05 | 0.21±0.06 | 21.68 | 29.35 | | | |
| | Hue | 49.03±11.56 | 67.03±13.42 | 23.58 | 20.02 | | | |
| Dorsum | Brightness | 68.68±30.84 | 80.19±42.36 | 44.9 | 52.82 | 20.58 | 3, 99 | <0.0001 |
| | Chroma | 0.22±0.05 | 0.21±0.05 | 22.63 | 26.39 | | | |
| | Hue | 43.94±10.89 | 62.07±12.84 | 24.79 | 20.68 | | | |
| Posterior | Brightness | 85.34±25.97 | 93.89±38.37 | 30.43 | 40.87 | 24.17 | 3, 99 | <0.0001 |
| | Chroma | 0.21±0.04 | 0.17±0.04 | 19.21 | 23.92 | | | |
| | Hue | 41.5±8.76 | 57.08±11.72 | 21.11 | 20.54 | | | |
| Tail | Brightness | 125.4±41.52 | 111.04±31.6 | 33.11 | 28.46 | 45.69 | 3, 99 | <0.0001 |
| | Chroma | 0.21±0.03 | 0.28±0.05 | 15.33 | 18.98 | | | |
| | Hue | 44.45±4.82 | 51.68±5.31 | 10.83 | 10.27 | | | |
| Cheek | Brightness | 82.55±63.81 | 33.14±22.48 | 77.29 | 67.81 | 13.93 | 3, 98 | <0.0001 |
| | Chroma | 0.15±0.06 | 0.09±0.05 | 36.80 | 50.26 | | | |
| | Hue | 42.48±12.64 | 34.36±13.96 | 29.75 | 40.64 | | | |
| Shoulder | Brightness | 111.55±81.36 | 58.49±47.73 | 72.93 | 81.60 | 9.84 | 3, 99 | <0.0001 |
| | Chroma | 0.16±0.03 | 0.13±0.05 | 21.72 | 36.52 | | | |
| | Hue | 48.24±16.27 | 60.55±46.57 | 33.73 | 76.91 | | | |
| Elbow | Brightness | 83.59±49.67 | 82.69±52.79 | 59.42 | 63.84 | 29.98 | 3, 99 | <0.0001 |
| | Chroma | 0.21±0.04 | 0.16±0.04 | 18.47 | 24.95 | | | |
| | Hue | 42.95±8.51 | 62.29±18.35 | 19.81 | 29.45 | | | |
| Flank | Brightness | 57.64±31.06 | 69.22±40.04 | 53.87 | 57.84 | 27.24 | 3, 99 | <0.0001 |
| | Chroma | 0.21±0.05 | 0.22±0.06 | 21.90 | 27.46 | | | |
| | Hue | 39.89±8.89 | 58.59±11.91 | 22.28 | 20.33 | | | |
| Chin | Brightness | 55.05±39.94 | 23.4±7.79 | 72.56 | 33.27 | 20.17 | 3, 99 | <0.0001 |
| | Chroma | 0.11±0.04 | 0.08±0.02 | 31.78 | 21.55 | | | |
| | Hue | 34.11±16.52 | 26.33±4.7 | 48.44 | 17.84 | | | |
| Throat | Brightness | 77.78±56.74 | 28.72±9.19 | 72.95 | 31.99 | 23.68 | 3, 98 | <0.0001 |
| | Chroma | 0.11±0.04 | 0.07±0.03 | 33.51 | 34.46 | | | |
| | Hue | 33.38±15.23 | 35.77±56.51 | 45.64 | 157.99 | | | |
| Chest | Brightness | 81.47±61.97 | 33.35±14.33 | 76.07 | 42.97 | 12.37 | 3, 98 | <0.0001 |
| | Chroma | 0.18±0.05 | 0.17±0.05 | 24.81 | 31.18 | | | |
| | Hue | 49.27±14.22 | 42.1±10.17 | 28.85 | 24.16 | | | |
| Belly | Brightness | 111.62±48.94 | 97.37±23.24 | 43.85 | 23.87 | 37.86 | 3, 98 | <0.0001 |
| | Chroma | 0.16±0.05 | 0.26±0.05 | 28.06 | 19.39 | | | |
| | Hue | 59.93±15.67 | 62.9±6.79 | 26.15 | 10.79 | | | |
| Abdomen | Brightness | 128.6±37.04 | 115.12±18.83 | 28.80 | 16.35 | 68.78 | 3, 98 | <0.0001 |
| | Chroma | 0.1±0.03 | 0.19±0.04 | 28.51 | 21.74 | | | |
| | Hue | 49.89±17.57 | 60.7±7.82 | 35.22 | 12.89 | | | |

**Note:** Mean (±SD) values and coefficients of variation (CV; %) are given for each component of colour (brightness, chroma, and hue) for different patches of the body.

interscapular region (paired *t* test: *t* = 2.65, *p* = 0.01, *n* = 49), posterior region (paired *t* test: *t* = 3.5, *p* = 0.001, *n* = 49), and flanks (paired *t* test: *t* = 3.13, *p* = 0.003, *n* = 49) (Fig. 4).

## Discussion

Melanism occurs in several lizard species, including those of the genus *Phymaturus*. Lobo and Nenda (2015) remark that melanism can be complete in the *patagonicus* group (southern species), but it is only present in the cephalic region in the *palluma* group (northern species). In the northern group, which includes *P. verdugo*, only males should be melanic because this is apparently determined by sexual dimorphism (Lobo and Nenda 2015). Cei and Videla (2003) remarked that the extent of black pigmentation depends on age and size of the specimens.

Our results do not support any of these statements. They show that melanism is present in both males and females and that it is not restricted to the cephalic region. Some females have melanic skin on the dorsum and flanks (e.g., Figs. 1E and 1H). Results from

spectrophotometry show that this species has a strong sexual dichromatism, but that it is not restricted to the cephalic region or based in the extension of melanism on the dorsum, as is commonly assumed. Males exhibited higher hue values than females in most patches, with peaks in the yellow and red parts of the spectrum, whereas females show peaks only in the red part of the spectrum (Fig. 3).

Melanism is not related with age or size, as some juveniles have proportionally larger dark areas than some adults (Fig. 1I). The different degrees of melanism observed by Cei and Videla (2003) for males, instead, reflect the chromatic variability in the species. Although some males show melanism as a black hood covering the head, neck, shoulders, and part of forelimbs (as reported in the original description), other males of similar size present melanism only on the head and neck (Figs. 1A–1D). In fact, the percentage of melanism in males varied widely, between 40% and 88%. The high CV in brightness for the shoulder and elbow also reflect this variability. Females have an even wider range of

**Fig. 3.** Reflectance curves for each body patch of males (black ×) and females (white ○) of the rocky lizard *Phymaturus verdugo*.
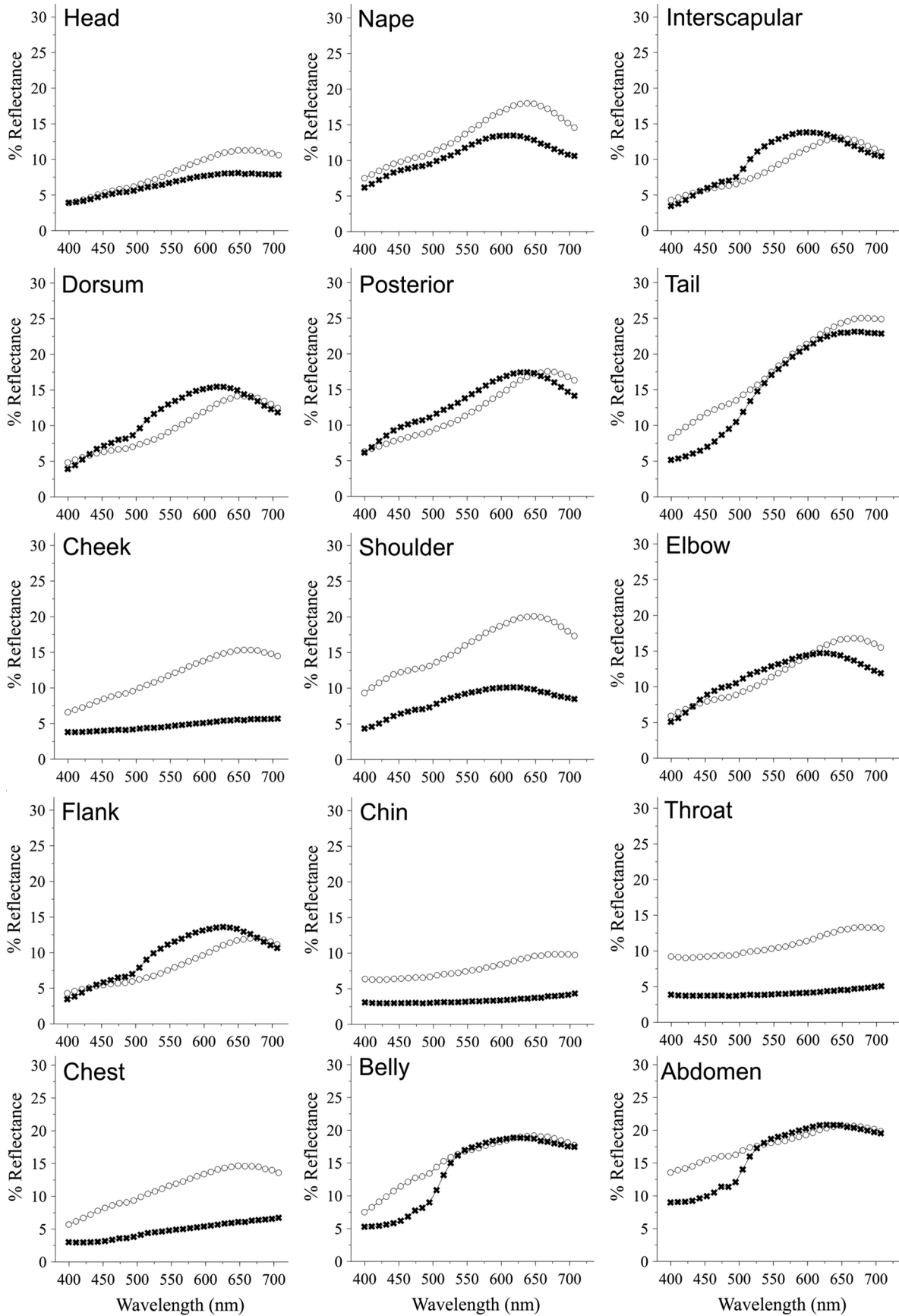
**Fig. 4.** Comparisons of each colour variable (brightness, chroma, and hue) between cold (light grey) and warm (dark grey) individuals of the rocky lizard *Phymaturus verdugo* for each body patch. H, head; N, nape; I, interscapular region; D, dorsum; P, posterior region; T, tail; C, cheek; S, shoulder; E, elbow; F, flank. Asterisks indicate patches that are significantly different between cold and warm lizards. Box limits indicate the 25th (lower) and 75th (upper) quartiles; the solid square within the box indicates the mean; the solid horizontal line indicates the median; whiskers indicate the last datum within 1.5 interquartile ranges of the box limits; solid squares beyond the whiskers represent atypical values, whereas open circles beyond the whiskers represent extreme values.
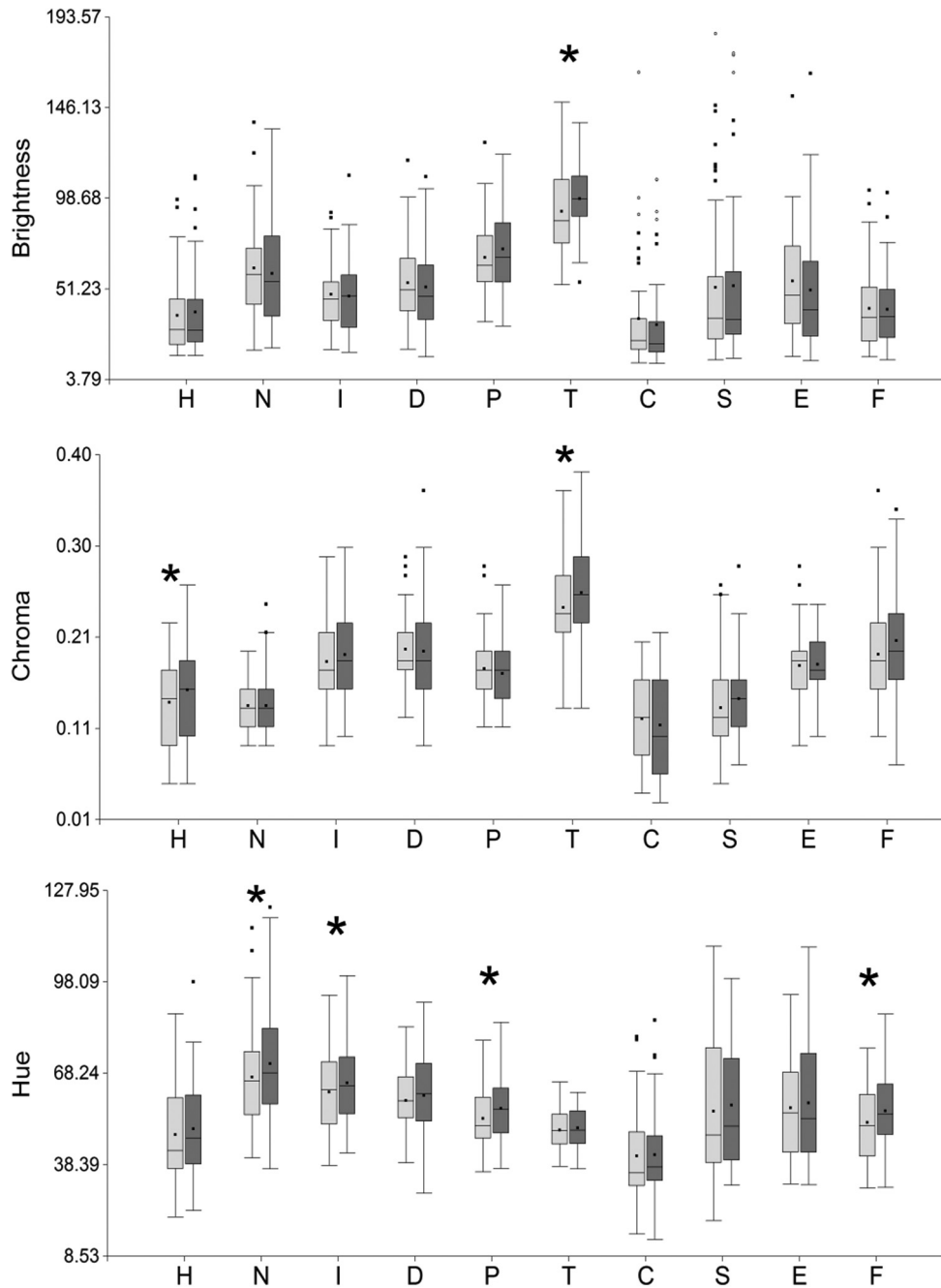


percentage of melanism, 11%–91%, with especially high CV for the cheek and shoulder (Table 1, Figs. 1E–1H). Although sexual dichromatism is frequently used to identify and describe species, our results highlight the need to use large samples to make accurate generalizations.

Explaining the chromatic variation should consider similarities with other species. Males of *P. verdugo* with the lowest melanism values are chromatically similar to males of *Phymaturus gynechlomus* Corbalán, Scolaro and Debandi, 2009 and *P. palluma*, which live farther north. There are low genetic differences between all three

species (V. Corbalán, unpublished data). This group needs to be revised with more data, which could be used to evaluate the possibility that it is a single species with morphological and chromatic variations based on location.

Studies of intra- and inter-specific variations in colouration along geographic gradients suggest that this variation is adaptive and is a response to local environmental conditions and selective agents (Stuart-Fox et al. 2004; Clusella-Trullas et al. 2007; Leaché et al. 2010; Farallo and Forstner 2012; Reguera et al. 2014). These studies propose that dark colouration might have evolved in

response to adverse conditions (low temperature and (or) high UV radiation) at high altitudes or latitudes. However, *P. verdugo* lizards vary in colouration and percentage of melanism even within the same population. This intrapopulation variability could be explained by other variables such as animal health, social status, signalling or cryptic advantages.

Our study evaluated the relationship between melanism and body condition (as an indirect measurement of body health), but we found no relationship, similar to a study of *Liolaemus xanthoviridis* Cei and Scolaro, 1980 (Escudero et al. 2016). The explanation for this was that body mass can vary greatly within and between activity seasons due to diet or pregnancy, among other factors. However, our study controlled for these possibilities. It was performed in the middle of the active period of lizards, all individuals were measured at the same time, and data from pregnant females were discarded. Hence, this cannot explain the lack of relationship in our data, which showed a weak relationship between body condition and sex. However, as body condition may be influenced by a diversity of traits, its relationship with melanism may be masked by other variables.

Melanism of individuals did not vary with body temperature. In the laboratory, individuals in captivity for over a year maintained their phenotypic characteristics, under the same conditions of light, substrate, and temperature (V. Corbalán, unpublished data), suggesting that melanism in *P. verdugo* has a significant genetic component. Instead, we detected shifts in darkness based on body temperature and activity. Several lizard species have darker dorsal colours when colder, suggesting that colour change has a role in thermoregulation (Norris 1967; Pearson 1977; Hoppe 1979; Sherbrooke et al. 1994; Rosenblum 2005). However, as with the Moorish Gecko (*Tarentola mauritanica* (Linnaeus, 1758)) (Vroonen et al. 2012), *P. verdugo* does not show lower brightness at low body temperatures, so thermoregulation in the first hours of the day is not mediated by colour changes in this species. Darkness differences at different temperatures or times of day are more related to variation in colour saturation (chroma) and colour peaks (hue) than total reflectance (brightness). Thus, the black head and the tail were more saturated when individuals were warm. Colourful patches such as interscapular and posterior regions and flanks showed peaks at lower wavelengths when animals were warm. Although most studies use only reflectance curves or brightness to demonstrate the effect of body temperature on colour, our results highlight the importance of chroma and hue. Variation in chroma and hue at different body temperatures may be related to crypsis — when animals are colder, their locomotor performance is reduced and they are more susceptible to predators (Angilletta et al. 2002).

The function of melanism in *P. verdugo* remains to be explained. In males, melanism may be related to social interaction. Ducrest et al. (2008) suggest that melanocortins enhance male sexual motivation and performance and have a positive effect on the production of sexual hormones. These peptide hormones promote aggressiveness (Ducrest et al. 2008) and predict dominance (Jawor and Breitwisch 2003). Future studies could test this hypothesis.

To summarize, our study has broken some paradigms regarding the genus *Phymaturus*. We demonstrated that melanism is not a character of sexual differentiation that occurs only in males. Some females were as melanic as some males and sexual dimorphism was present all over the body, not only in the cephalic region. We also demonstrated that there is no relationship between melanism and SVL, indicating that, at least in these populations, melanism does not increase with age. Hence, our human perception of colour, as well as working with small samples, can lead to misidentification or erroneous species descriptions. In terms of colour variables, hue and chroma may be more important than brightness early in the day when animals are still cold; variation is probably due to crypsis, not thermoregulation. There-

fore, darker skin in the morning is not only related to heat absorption.

The evolution of alternative phenotypes can have significant impacts on biology (Escudero et al. 2016), an approach that may begin to explain how chromatic variability evolved in the genus *Phymaturus*. Further explorations of this should evaluate a wide range of variables that may be related to melanism such as heating rates, crypsis, sexual selection, male aggression and interaction, immune response, and UV protection.

## Acknowledgements

## References

Angilletta, M.J., Niewiarowski, P.H., and Navas, C.A. 2002. The evolution of thermal physiology in ectotherms. J. Therm. Biol. **27**: 249–268. doi:10.1016/S0306-4565(01)00094-8.

Bagnara, J.T., and Hadley, M.E. 1973. Chromatophores and color change: the comparative physiology of animal pigmentation. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Baird, T.A., Fox, S.F., and McCoy, J.K. 1997. Population differences in the roles of size and coloration in intra- and intersexual selection in the collared lizard, *Crotaphytus collaris*: influence of habitat and social organization. Behav. Ecol. **8**: 506–517. doi:10.1093/beheco/8.5.506.

Böhm, M., Wolff, I., Scholzen, T.E., Robinson, S.J., Healy, E., Luger, T.A., Schwarz, T., and Schwarz, A. 2005. α-Melanocyte-stimulating hormone protects from ultraviolet radiation-induced apoptosis and DNA damage. J. Biol. Chem. **280**: 5795–5802. doi:10.1074/jbc.M406334200. PMID:15569680.

Boretto, J.M., Cabezas-Cartes, F., and Ibargüengoytía, N.R. 2015. Energy allocation to growth and reproduction in a viviparous lizard endemic to the highlands of the Andes, Argentina. J. Zool. (Lond.), **297**: 77–86. doi:10.1111/jzo.12245.

Boretto, J.M., Cabezas-Cartes, F., and Ibargüengoytía, N.R. 2018. Slow life histories in lizards living in the highlands of the Andes Mountains. J. Comp. Physiol. B, **188**(3): 491–503. doi:10.1007/s00360-017-1136-z. PMID:29150716.

Capitanelli, R.G. 1999. Climas de la Provincia de Mendoza. Meridiano, **7**: 29–40.

Carretero, M.A. 2002. Sources of colour pattern variation in Mediterranean *Psammodromus algirus*. Neth. J. Zool. **52**: 43–60. doi:10.1163/156854202760405177.

Cei, J.M., and Videla, F. 2003. A new *Phymaturus* species from volcanic cordilleran mountains of the south-western Mendoza Province, Argentina (Liolaemidae, Iguania, Lacertilia, Reptilia). Boll. Mus. Reg. Sci. Nat., Torino, **20**: 291–314.

Chai, B., Li, J., Zhang, W., Newman, E., Ammori, J., and Mulholland, M.W. 2006. Melanocortin-4 receptor-mediated inhibition of apoptosis in immortalized hypothalamic neurons via mitogen-activated protein kinase. Peptides, **27**: 2846–2857. doi:10.1016/j.peptides.2006.05.005. PMID:16806584.

Clusella-Trullas, S., van Wyk, J.H., and Spotila, J.R. 2007. Thermal melanism in ectotherms. J. Therm. Biol. **32**: 235–245. doi:10.1016/j.jtherbio.2007.01.013.

Clusella-Trullas, S., Terblanche, J.S., Blackburn, T.M., and Chown, S.L. 2008. Testing the thermal melanism hypothesis: a macrophysiological approach. Funct. Ecol. **22**: 232–238. doi:10.1111/j.1365-2435.2007.01377.x.

Clusella-Trullas, S., van Wyk, J., and Spotila, J. 2009. Thermal benefits of melanism in cordylid lizards: a theoretical and field test. Ecology, **90**: 2297–2312. doi:10.1890/08-1502.1. PMID:19739391.

Ducrest, A.L., Keller, L., and Roulin, A. 2008. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends Ecol. Evol. **23**: 502–510. doi:10.1016/j.tree.2008.06.001. PMID:18644658.

Endler, J. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biol. J. Linn. Soc. **41**: 315–352. doi:10.1111/j.1095-8312.1990.tb00839.x.

Escudero, P.C., Minoli, I., González Marín, M.A., Morando, M., and Avila, L.J. 2016. Melanism and ontogeny: a case study in lizards of the *Liolaemus fitzingerii* group (Squamata: Liolaemini). Can. J. Zool. **94**(3): 199–206. doi:10.1139/cjz-2015-0112.

Farallo, V.R., and Forstner, M.R.J. 2012. Predation and the maintenance of color polymorphism in a habitat specialist Squamate. PloS ONE, **7**: e30316. doi:10.1371/journal.pone.0030316. PMID:22295080.

Geen, M., and Johnston, G. 2014. Coloration affects heating and cooling in three color morphs of the Australian bluetongue lizard, *Tiliqua scincoides*. J. Therm. Biol. **43**: 54–60. doi:10.1016/j.jtherbio.2014.04.004. PMID:24956958.

Germano, D.J., and Williams, D.F. 2007. Ontogenetic and seasonal changes in coloration of the blunt-nosed leopard lizard (*Gambelia sila*). Southwest. Nat. **52**: 46–53. doi:10.1894/0038-4909(2007)52[46:OASCIC]2.0.CO;2.

Getting, S.J. 2006. Targeting melanocortin receptors as potential novel therapeutics. Pharmacol. Ther. **111**: 1–15. doi:10.1016/j.pharmthera.2005.06.022.

Hadley, M.E. 1997. Comparative aspects of non-mammalian pigmentation: regulation of pigment cells. Pigment Cell. Res. **10**: 114.

Hoppe, D.M. 1979. The influence of color on behavioral thermoregulation and hydroregulation. *In* The behavioral significance of color. *Edited by* E.H. Burtt, Jr. Garland STOM Press, New York. pp. 35–62.

InfoStat. 2009. InfoStat version 2009. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina.

Jawor, J.M., and Breitwisch, R. 2003. Melanin ornaments, honesty, and sexual selection. Auk, **120**: 249–265. doi:10.1642/0004-8038(2003)120[0249:MOHASS]2.0.CO;2.

Leaché, A.D., Helmer, D., and Moritz, C. 2010. Phenotypic evolution in high-elevation populations of western fence lizards (*Sceloporus occidentalis*) in the Sierra Nevada Mountains. Biol. J. Linn. Soc. **100**: 630–641. doi:10.1111/j.1095-8312.2010.01462.x.

Legendre, P. 2015. lmodel2: Model II Regression. R package version 1.7-2 [computer program]. Available from https://CRAN.R-project.org/package=lmodel2.

Lele, S.R., Keim, J.L., and Solymos, P. 2017. Resource selection (probability) functions for use-availability data. Available from https://github.com/psolymos/ResourceSelection.

Lobo, F., and Nenda, S.J. 2015. Discovery of two new species of *Phymaturus* (Iguania: Liolaemidae) from Patagonia, Argentina, and occurrence of melanism in the *patagonicus* group. Cuad. Herpetol. **29**: 5–25.

Macedonia, J.M., Brandt, Y., and Clark, D.L. 2002. Sexual dichromatism and differential conspicuousness in two populations of the common collared lizard (*Crotaphytus collaris*) from Utah and New Mexico, USA. Biol. J. Linn. Soc. **77**: 67–85. doi:10.1046/j.1095-8312.2002.00092.x.

McGuire, J.A. 1996. Phylogenetic systematics of crotaphytid lizards (Reptilia: Iguania: Crotaphytidae). Bull. Carn. Mus. Nat. Hist. **32**: 1–143.

Moreno Azócar, D.L., Bonino, M.F., Perotti, M.G., Schulte, J.A., Abdala, C.S., and Cruz, F.B. 2016. Effect of body mass and melanism on heat balance in *Liolaemus* lizards of the *goetschi* clade. J. Exp. Biol. **219**: 1162–1171. doi:10.1242/jeb.129007. PMID:26896550.

Morrison, R.L., Rand, M.S., and Frost-Mason, S.K. 1995. Cellular basis of color differences in three morphs of the lizard *Sceloporus undulatus erythrocheilus*. Copeia, **1995**: 397–408. doi:10.2307/1446903.

Norris, K.S. 1967. Color adaptation in desert reptiles and its thermal relationships. *In* Lizard ecology: a symposium. *Edited by* W.W. Milstead. University of Missouri Press, Columbia. pp. 162–229.

Ortíz Maldonado, A. 2001. Distribución geográfica de los elementos meteorológicos principales y adversidades de Mendoza. Triunfar, Córdoba, Argentina.

Pearson, O.O. 1977. The effect of substrate and of skin color on thermoregulation of a lizard. Comp. Biochem. Physiol. **58**: 353–358. doi:10.1016/0300-9629(77)90154-2.

Peig, J., and Green, A.J. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos, **118**: 1883–1891. doi:10.1111/j.1600-0706.2009.17643.x.

Peig, J., and Green, A.J. 2010. The paradigm of body condition: a critical reappraisal of current methods based on mass and length. Funct. Ecol. **24**: 1323–1332. doi:10.1111/j.1365-2435.2010.01751.x.

Porter, W.P., and Gates, D.M. 1969. Thermodynamic equilibria of animals with environment. Ecol. Monogr. **39**: 227–244. doi:10.2307/1948545.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from https://www.r-project.org.

Reguera, S., Zamora-Camacho, F.J., and Moreno-Rueda, G. 2014. The lizard *Psammodromus algirus* (Squamata: Lacertidae) is darker at high altitudes. Biol. J. Linn. Soc. **112**: 132–141. doi:10.1111/bij.12250.

Rosenblum, E.B. 2005. The role of phenotypic plasticity in color variation of Tularosa Basin lizards. Copeia, **2005**: 586–596. doi:10.1643/CP-04-154R1.

Schindelin, J., Rueden, C.T., Hiner, M.C., and Eliceiri, K.W. 2015. The ImageJ ecosystem: an open platform for biomedical image analysis. Mol. Reprod. Dev. **82**: 518–529. doi:10.1002/mrd.22489. PMID:26153368.

Sherbrooke, W.C., Castrucci, A.M., and Hadley, M.E. 1994. Temperature effects on in vitro skin darkening in the Mountain spiny lizard, *Sceloporus jarrovii*: a thermoregulatory adaptation? Physiol. Zool. **67**: 659–672. doi:10.1086/physzool.67.3.30163763.

Smith, S.D. 2014. Quantifying color variation: improved formulas for calculating hue with segment classification. Appl. Plant Sci. **2**(3): 1300088. doi:10.3732/apps.1300088.

Stuart-Fox, D., and Moussalli, A. 2008. Selection of social signalling drives the evolution of chameleon colour change. PLoS Biol. **6**: 22–29. doi:10.1371/journal.pbio.0060025.

Stuart-Fox, D.M., Moussalli, A., Johnston, G., and Owens, I.P.F. 2004. Evolution of color variation in dragon lizards: quantitative tests of the role of crypsis and local adaptation. Evolution, **58**: 1549–59. doi:10.1111/j.0014-3820.2004.tb01735.x. PMID:15341157.

Trombotto Liaudat, D., Penas, P., and Aloy, G. 2014. Impact of volcanic processes on the cryospheric system of the Peteroa Volcano, Andes of southern Mendoza, Argentina. Geomorphology, **208**: 74–87. doi:10.1016/j.geomorph.2013.11.016.

Vroonen, J., Vervust, B., Fulgione, D., Maselli, V., and Van Damme, R. 2012. Physiological colour change in the Moorish gecko, *Tarentola mauritanica* (Squamata: Gekkonidae): effects of background, light, and temperature. Biol. J. Linn. Soc. **107**: 182–191. doi:10.1111/j.1095-8312.2012.01915.x.

Walton, B.M., and Bennett, A.F. 1993. Temperature-dependent color change in Kenyan chameleons. Physiol. Zool. **66**: 270–287. doi:10.1086/physzool.66.2.30163690.