

Is it all about size? Dismantling the integrated phenotype to understand species coexistence and niche segregation

Carolina Reyes-Puig^{1,2,3,4}  | Urtzi Enríquez-Urzelai⁵  | Miguel A. Carretero^{1,2,3}  | Antigoni Kaliontzopoulou⁶ 

¹CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, Vairão, Portugal; ²BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, Vairão, Portugal; ³Departamento de Biología, Faculdade de Ciências, Universidade do Porto, Porto, Portugal; ⁴Instituto de Biodiversidad Tropical IBIOTROP, Museo de Zoología, Colegio de Ciencias Biológicas y Ambientales COCIBA, Universidad San Francisco de Quito, Quito, Ecuador; ⁵Czech Academy of Sciences, Institute of Vertebrate Biology, Brno, Czech Republic and ⁶Department of Evolutionary Biology, Ecology and Environmental Sciences, and Biodiversity Research Institute (IRBio), Universitat de Barcelona, Barcelona, Catalonia, Spain

Correspondence

Carolina Reyes-Puig
Email: creyesp@usfq.edu.ec

Funding information

Asociación Española de Ecología Terrestre (AEET); Universidad San Francisco de Quito, Museo de Zoología & Laboratorio de Zoología Terrestre, Instituto de Biodiversidad Tropical IBIOTROP, Grant/Award Number: 17162; Ramón y Cajal research grant co-funded by the Spanish State Research Agency and the European Social Fund, Grant/Award Number: RYC2019-026688-I/AEI/10.13039/501100011033; FEDER Funds through the Operational Competitiveness Factors Program—COMPETE and National Funds through FCT—Foundation for Science and Technology within the scope of the projects, Grant/Award Number: PTDC/BIA-EVL/28090/2017-POCI-01-0145-FEDER-028090 and PTDC/BIA-CBI/28014/2017-POCI-01-0145-FEDER-028090; Russell E. Train Education for Nature Initiative from WWF

Handling Editor: Timothy Higham

Abstract

1. Niche segregation is a mechanism by which competition between coexisting species is reduced. The ecological niche is a multidimensional space shaped by the conditions and resources that enable the existence of species.
2. We conducted comprehensive univariate and multidimensional analyses of phenotypic traits encompassing morphology, functional performance and ecophysiology, to investigate which phenotypic traits contribute to niche segregation and overlap in two coexisting green lizard species.
3. Our analyses revealed that the main driver of niche segregation was body size. However, when considering size-corrected phenotypic spaces, ecophysiological traits were still distinct, with little overlap between co-occurring species. Such differentiation was linked mainly to preferred temperature variance and water loss, highlighting the importance of thermal and hydric niche segregation.
4. Some particular traits such as limb length also contributed to niche segregation being maintained in the morphological space, even when the effect of size was accounted for. Instead, the phenotypic space of functional performance traits (i.e. bite force, locomotor performance) showed the greatest overlap between the two species, thus, less niche segregation was observed, once size effects were removed. Therefore, functional performance traits contribute in a minor proportion to the effective niche segregation between the two species. In light of our results, the most adequate perspective to understand niche segregation in coexisting species is through a multidimensional approach in differentiated phenotypic spaces. Our dismantling of phenotypic traits allowed us to identify niche areas in which trait overlap occurs and others that promote niche segregation, considering or not size effects.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial License](#), which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2024 The Author(s). *Functional Ecology* published by John Wiley & Sons Ltd on behalf of British Ecological Society.

5. Our results suggest that differential use of structural and functional niche space promotes effective niche segregation, potentially reducing direct competition between species. We highlight the importance of studies that include the combination of several phenotypic traits that, as a whole, provide insights to better understand the mechanisms by which coexisting organisms exploit differentiated resources in multidimensional spaces.

KEY WORDS

ecological niche, ecophysiology, functional traits, hyperspace, niche dimensions, performance

1 | INTRODUCTION

The factors promoting species coexistence have puzzled scientists for decades (Amarasekare, 2002; Armstrong & McGehee, 1976; Gravel et al., 2011; Pigot et al., 2016; Zobel, 1992). This occurs when different species share the same habitat or ecosystem without one species being displaced by the presence of the other (Gravel et al., 2011; Valladares et al., 2015). In classical niche theory, coexistence is allowed when each species utilizes a unique combination of biotic and abiotic resources, which are limited regarding the populations of such species, which translates into having different niches (Colwell & Rangel, 2009; Grinnell, 1917; Hutchinson, 1978). Under the same theory, if resources are limited and niches overlap, the less efficient competitor will be displaced (Hutchinson, 1978; Schoener, 1974). As such, species coexistence is typically achieved through niche segregation (Gravel et al., 2011; Tokeshi, 2009), which involves the differentiation of resource use between species that co-occur in a given area (Schoener, 1974; Valladares et al., 2015). Niche segregation facilitates species coexistence by minimizing competitive interactions for resources, thus reducing the risk of one species excluding the other (Larson, 1984; Pianka & Huey, 1978; Valladares et al., 2015). Niche segregation could reduce competition among species by fulfilling its resource requirements either at different times (temporal segregation) or by causing each species to specialize in a particular area of the multivariate niche space (including time as part of it) (Castro-Arellano & Lacher, 2009; Prins et al., 2006; Smart & Gee, 1979).

The ecological niche can be summarized as the multidimensional space shaped by the conditions and resources that allow species to exist (Colwell & Rangel, 2009; Hutchinson, 1978), that is an integrated set of conditions that determine species' survival and reproductive success (Kearney, 2006). Importantly, a comprehensive view of the niche considers it a property of species—and not merely a description of the habitat they occupy—which emerges through the interplay between environmental conditions and species phenotype (i.e. behaviour, morphology, physiology and life history) (Colwell & Rangel, 2009; Elton, 1927; Hutchinson, 1957; Kearney, 2006). Implicitly, phenotypes are also multidimensional and interact with the environment in order to determine species' niches. Among phenotypic traits, morphology influences—among other things—how organisms exploit their structural habitat (e.g.

foraging, sheltering, escaping) (Kaliotzopoulou et al., 2012; Peterson & Husak, 2006; Williams, 1983). Morphology also affects locomotor performance (e.g. sprint speed, manoeuvrability) mediated by the surrounding environment and anatomical adaptations (e.g. elasticity, joint flexibility) (Farley, 1997; Farley & Ko, 1997; Gomes et al., 2016; Goodman et al., 2008; Vasilopoulou-Kampitsi, 2020). Also, it is linked to the trophic and social axes of the niche through head proportions and bite force (Gomes et al., 2018; Gomes et al., 2020; Huyghe et al., 2005; Lappin & Husak, 2005; McBrayer & White, 2002). Furthermore, organisms' physiology (e.g. heat and water exchange) is related to the surrounding environment (Kearney, 2006; Mi et al., 2022), and in terrestrial organisms, it is associated with morphological and functional adaptations to minimize dehydration and to enhance heat interchange (Angilletta Jr., 2009; de la Vega & Schilman, 2017; Eynan & Dmi'el, 1993; Kearney & Porter, 2009; Mautz, 1980; Morales & Giannini, 2010). However, it is crucial to understand that while morphology influences different aspects of an organism's behaviour and performance with its surrounding habitat, it does not mean that it exclusively defines the ecological niche. Rather, morphology is tightly connected to niche utilization, regarded as a proxy indicator of niche segregation. Thus, describing multidimensional phenotypes—and how and to what extent they overlap—can shed light on the mechanisms that facilitate niche segregation in coexisting species (Garland Jr & Losos, 1994; Vasil'ev, 2021).

The overall dimensions of an organism and the distinct anatomical features of which it is composed represent its size. In this regard, body size is a key phenotypic trait that can affect each of the niche axes through allometric and biophysical effects. That is, body size is tightly linked to each of the aforementioned morphological, functional performance and physiological traits (Angilletta Jr et al., 2004; Sears & Angilletta Jr, 2004). Body size has a significant impact on functional performance, since muscle strength is positively related to body size and mass (Aasa et al., 2003; Irschick et al., 2008). For example, relative head and limb sizes are well known to contribute to variation in bite and locomotor performance (Herrel et al., 2001; Huyghe et al., 2005). Similarly, there is a well-established relationship between body size and thermal physiology (Angilletta Jr., 2009; Baudier & O'Donnell, 2018; Streinzer et al., 2016). For instance, larger organisms tend to have higher tolerance to higher temperatures and smaller ones tend to

have lower minimum critical temperature values, also, variation in body size may be related to regional heterothermic capacity (Baudier & O'Donnell, 2018; Olalla-Tárraga et al., 2006; Webber & McGuire, 2022). In addition, smaller animals usually have higher per gram relative metabolic rates due to lower thermal inertia (Angilletta Jr., 2009; Kearney & Porter, 2009). Finally, larger ectotherms exhibit a lower surface area-to-volume ratio, thus reducing water loss (Chown & Klok, 2003). Thus, in this context, body size is particularly relevant since it covaries with most phenotypic traits (Ayers & Shine, 1997; Carlson et al., 2008; Tobalske & Dial, 2000), and it may therefore serve as a primary mechanism for species differentiation, facilitating niche segregation and promoting species coexistence. Still, residual trait differentiation between coexisting species may further contribute to niche segregation, after size is accounted for.

With this integrated multivariate approach, we intend to identify the main directions of species' differentiation pinpointing those that could allow their coexistence. Here, we used as our study model syntopic populations (i.e. populations of species that occur at the same time in a particular locality) of *Timon lepidus* and *Lacerta schreiberi* in Northern Portugal (Brito, Paulo, & Crespo, 1998; Enriquez-Urzelai et al., 2022; Loureiro et al., 2008). Both species are considerably different in body size, being *T. lepidus* larger than *L. schreiberi* (Brito, Luis, et al., 1998; Brito, Paulo, & Crespo, 1998; Marco & Pollo, 1993; Mateo & Castanet, 1994), and they frequently share habitats. However, *T. lepidus* tends to show varied spatial patterns in Mediterranean habitats such as shrublands and other more semi-arid areas (Renet et al., 2022). It usually moves on rocky substrates and open areas, using rock holes as shelter (Ferreira et al., 2016). Other studies have also detected the presence of *T. lepidus* in structures of anthropogenic origin, reflecting the adaptability of the species to different microhabitat conditions (Delgado & Gómez, 2016). On the other hand, *L. schreiberi* prefers microhabitats with an increased presence of shrubs and bushes (Salvador, 1988), where soil and vegetation characteristics are important for the occurrence of this species (Brito, Luis, et al., 1998; Brito, Paulo, & Crespo, 1998). Given its elevated dependence on vegetation, the relative humidity of the surrounding environment is a potential factor to be examined. Regarding their trophic niche, there is some evidence that both species consume similar items such as beetles, grasshoppers, ants and spiders among others (Pérez Mellado, 1982; Salvador, 1988; Salvidio et al., 2006). Thus, these species are appropriate for investigating phenotypic differentiation in coexisting species, and for understanding the contribution of body size to niche segregation. Specifically, we aim to provide a comprehensive framework for examining phenotypic space partitioning between coexisting species to infer the potential drivers of niche segregation, combining morphological, performance and physiological traits. Through this approach, we want to clarify the interplay between morphology and function, with special emphasis on the relationship between body size and different phenotypic traits. We hypothesize the existence of differences in morphology, functional performance,

thermal and water balance between both species. Since body size is closely linked to allometric trait relationships, we predict that the effect of body size will be significant in all traits, with the greatest influence on morphology. Since *T. lepidus* and *L. schreiberi* are known to occupy relatively different microhabitats in particular areas (Marco & Pollo, 1993; Mateo & Cheylan, 1997), we expect niche segregation to be maintained once the effect of body size is removed from performance and ecophysiological traits. Likewise, we expect greater phenotypic overlap between species when the body size effect is removed and greater segregation when it is included. By investigating the integration of phenotypic axes, we expect to gain insights on the mechanisms and traits underlying the differences between coexisting species. Thus, in this way, we anticipate identifying phenotypic traits that are species-specific and not a consequence of body size scaling and to gain a better understanding of the available phenotypic components that may contribute to niche segregation.

2 | MATERIALS AND METHODS

2.1 | Study species, sampling and animal husbandry

The ocellated green lizard *Timon lepidus* (Daudin, 1802) is a European reptile typical of western Mediterranean regions, mainly distributed in northeastern Italy, southern France, and the Iberian Peninsula, from sea level to 2000 meters of elevation (Mateo, 2017; Mateo & Cheylan, 1997). The Schreiber's green lizard *Lacerta schreiberi* Bedriaga, 1879 is an Atlantic species, endemic to Western Iberian Peninsula, distributed from sea level to 2100 meters of elevation (Brito, Paulo, & Crespo, 1998; Marco & Pollo, 1993). Despite their overall morphological resemblance, the two species are distinguished mainly by their difference in body size, *T. lepidus* being one of the largest species of the green lizard clade in Europe reaching more than 240 mm of snout to vent length (SVL), while *L. schreiberi* is about 130 mm of SVL (Brito, Luis, et al., 1998; Mateo, 2017). Both species are relatively flexible ecologically; however, *T. lepidus* is generally considered to have a broad plasticity for habitat preferences (Galán, 2003; Llorente et al., 1995), while *L. schreiberi* is very linked to humid environments (e.g. moist deciduous forests, heathlands and locations close to running streams; Brito, Paulo, & Crespo, 1998).

In May and June 2022, we sampled individuals of *T. lepidus* and *L. schreiberi* from a sympatric locality in northern Portugal (Castro de São Paio; 41.31225°N, 8.737074°W; 12 m a.s.l.). Since both species are sexually dimorphic, we restricted our sampling to adult males to avoid sex-, pregnancy- and ontogeny-related biases (Carretero et al., 2005). We collected a total of 26 males of *T. lepidus* and 16 *L. schreiberi* by noosing as described by Sillero and García-Muñoz (2010). Once the specimens were collected, they were transported to the laboratory of CIBIO. Individuals were assigned a unique code with non-toxic marker paint to identify them during the experiments and were individually housed in terraria with

water ad libitum with no food. Between experiments, the terraria were cleaned and provided with clean water, and the air temperature maintained at 25°C. All animals rested one day before starting the experiments.

All the processes of collection, management and experimentation with green lizards were carried out with the collection and research permits LICENÇA No. 344-348/2022/CAPT obtained from the Instituto da Conservação da Natureza e Florestas. In addition, the Órgão Responsável pelo Bem-Estar dos Animais (ORBEA) was informed about the practices and details of the project.

2.2 | Experimental procedures and data analysis

2.2.1 | Replication statement

Our study involves co-occurring populations of two species of green lizards. Specifically, we examined 26 individuals of *T. lepidus* and 16 individuals of *L. schreiberi*, allowing us to analyse the scale of inference at the population level within each species.

2.2.2 | Morphology

We recorded the following linear measurements in all collected individuals, using electronic callipers with a precision of 0.01 mm: snout-to-vent length (SVL), trunk length (TRL), head length (HL), head width (HW), head height (HH), fore limb length (FLL) and hind limb length (HLL). The individuals were also weighed using a precision balance (± 0.0001 g; Sartorius M-Pact AX224, Sartorius AG, Goettingen, Germany). We used SVL as a proxy of body size. To obtain a single metric for head size (HS), we calculated the geometric mean of HL, HW and HH. To investigate morphological differences between species, we used ANOVA comparisons for SVL, and ANCOVAs for the other morphological traits with SVL as covariate and species as factor. For downstream analyses (see further on), we obtained size-corrected variables for each species by regressing each morphological trait on body size and we used the residuals from this regression for further analyses. The decision on using SVL for size correction was based on the AIC values observed after including body size or body weight as a covariate (Table SM1.1). The size-corrected morphological variables are abbreviated in figures and tables as res.TRL, res.HS, res.FLL and res.HLL. Measurement data collection lasted 2 h for each set of 8 specimens and were taken on the first day.

All ANOVA and ANCOVA comparisons for morphology as well as the performance and ecophysiology analyses detailed below were evaluated for significance using residual randomization procedures with 1000 permutation cycles as implemented in the package 'RRPP' (Collyer & Adams, 2019). We used this approach since does not rely on parametric assumptions and is suitable for relatively small sample sizes (Collyer & Adams, 2019). For generalized linear models we used the 'lme4' R package (Bates et al., 2014),

and 'tidyverse' (Wickham et al., 2019) for data cleaning, wrangling and visualization.

2.2.3 | Locomotor performance

We started the locomotor performance experiment on the morning of the first day, therefore, the lizards were in conditions most similar to the field. Racetrack experiments lasted between in total 4 and 5 h for each set of 8 specimens, this means that each lizard rested at least 20 min in thermal chambers after each trial. Thus, no lizard performed sequential runs on the racetracks (see details in SM2). We followed standard procedures for locomotor performance experiments (Gomes et al., 2018; Herrel et al., 2000). We examined locomotor performance in two different ecologically relevant settings (Figure 1a,b). A straight racetrack to assess maximum sprint speed (MaxSPR) (Figure 1a) and a double L-racetrack to assess manoeuvrability (MNV) (Figure 1b). Details on racetrack specifications and exact experimental conditions are available in SM2. Each individual performed the runs in each racetrack three times to guarantee that maximal locomotor performance was recorded. To motivate lizards to run, we chased them during the trials in both racetracks. We filmed all trials with a digital camera (specifications in SM2). We excluded from analyses all the trials in which the lizards completely stopped or turned to face the researchers.

For MaxSPR, we calculated the maximum instantaneous speed (evaluated between consecutive frames, Gomes et al., 2018) across all trial repetitions. We considered the following variables to describe MNV: (i) success of the turn, considering as unsuccessful any turns in which the lizard collided or climbed the walls, (ii) maximal speed during the path and turn; (iii) maximum bending angle of the trunk and head during a turn; and (iv) manoeuvring strategy (MS), modified from the categories proposed by Vasilopoulou-Kampitsi (2020): MS0 trials in which lizards crashed against the walls and therefore, unsuccessful turns; MS1 trials in which lizards completed the turns stopping before the turns; MS2 trials in which the lizards completed the turn without stopping. All data on locomotor performance were log-transformed prior to analyses.

To explore differences in maximum sprint speed (MaxSPR), we performed an ANCOVA including body size as a covariate and species as a factor. To explore how other morphological traits biomechanically associated with locomotor capacity contribute to variation between species in sprint speed, we performed ANCOVA comparisons on sprint speed using size-corrected limb traits and species as predictors. To evaluate turning success as a measure of manoeuvrability, we used a chi-squared proportions test, implemented in the function prop.test of the 'stats' R-package (R Core Team, 2024) to assess whether the proportions of turning success are significantly different between the two species. To investigate the combined effect of body size and running speed while in the double-L racetrack, we performed binomial generalized mixed models with turning success as the response variable, body size and manoeuvring strategy (i.e. whether the lizard stopped before the turn

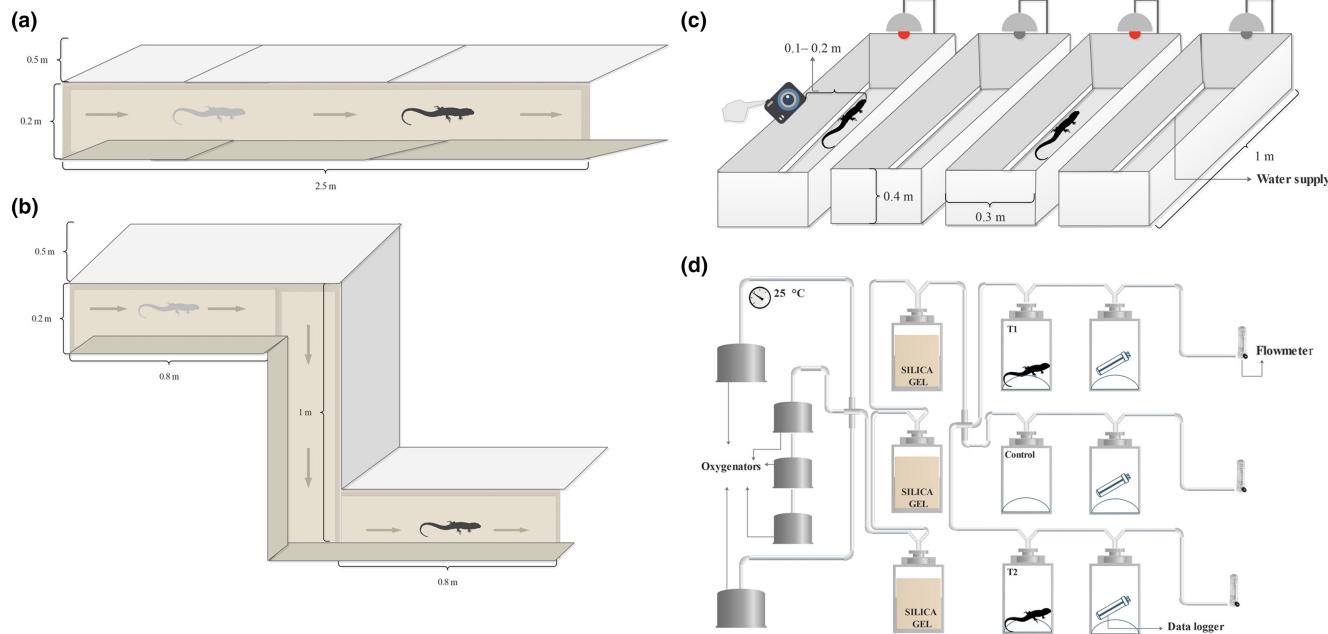


FIGURE 1 Schematic representation of the experiments carried out to record phenotypic traits. (a) Straight racetrack, (b) double-L racetrack (i.e. a straight surface of 0.8 m, an angle of 90°, a straight surface of 1 m, a second angle of 90° and a final straight surface of 0.8 m), (c) thermogradient chambers and (d) evaporative water loss quantification system.

or not) as interacting explanatory factors and maximum speed in the double-L corridor as an additive factor. For downstream analyses (as detailed later on), we obtained size-corrected variables by regressing each locomotor trait on body size and extracting the residuals from this regression for further analyses. We used body size to correct manoeuvrability traits based on the AIC values observed after including body size or body weight as a covariate (Table SM1.1). The size-corrected locomotor variables are abbreviated in figures and tables as res.MaxSPR, res.HA, res.Max_L (for each abbreviation of locomotion traits see SM2).

2.2.4 | Bite force

We measured bite force (BF) with an isometric Kistler force transducer, using standard procedures (Herrel et al., 2001; SM2). Bite force measurements took 2 h for eight specimens during the end of first day of experiments, after the lizards had rested for at least 2 h from the previous performance experiments. We put two marks on the edge of the plates to delimit where the lizards should bite to ensure an equal distance and thus standardize the point of effort of the bite force (Gomes et al., 2020). We tested each individual five times to ensure that we quantified the maximal bite force of each lizard. We retained the maximum bite force (in N) per lizard and log-transformed the data before statistical analyses. At the end of the first day of experiments, the lizards rested for 48 h with water ad libitum (see details in SM2).

We used an ANOVA to investigate the differences in bite force between species and ANCOVA including body weight and head size as covariate and species as factor, considering all interaction terms.

For subsequent analyses (see further on), we acquired residuals of size-corrected bite force values by conducting a regression between bite force on body weight (res.BF). We based this decision on the AIC values observed once body size or body weight were accounted for (Table SM1.1). Finally, we performed an ANCOVA between size-corrected bite force with size-corrected head size as covariate and species as factor to explore if head size had an effect on size-corrected bite force.

2.2.5 | Preferred body temperatures

To quantify the thermal preference of individuals, we conducted selected temperature experiments between 9 h and 17:30 h on the fourth day, as the second and third days were intended for the lizards to rest and acclimatize to the thermogradient. We measured body temperatures every half an hour, resulting in 19 temperature measurements per individual. To measure body temperatures, we used a FLIR T335 thermal camera (Flir Systems Inc., Wilsonville, Oregon, USA). Details on the preparation of the lizards prior to the experiments, as well as specifications and dimensions of the experimental arena and the instrumentation used are available in SM2 and Figure 1c.

To summarize the thermal preference of each species, we calculated the mean (i.e. preferred, T_{pref}), median, minimum, maximum, variance ($T_{\text{pref}}V$) and set point range of body temperature per individual (25%–75% quartile) (Angilletta Jr., 2009). To evaluate differences in preferred temperatures (T_{pref} and $T_{\text{pref}}V$) between species we performed an ANOVA with species as the explanatory variable. In a second ANCOVA model, body weight was added as a

covariate to incorporate the effect of size, as used in other similar studies (Barroso et al., 2020) and based in the AIC values displayed on models with body size or body weight (Table SM1.1). For downstream analyses of species differentiation across a size-free multivariate phenotypic space, we obtained the residual values from a regression of T_{pref} and $T_{\text{pref}}V$ on body weight (res. T_{pref} and res. $T_{\text{pref}}V$, respectively).

2.2.6 | Water loss

Water loss experiments were conducted on the fifth day, after the lizards had rested for at least 15 h from the previous experiment (thermogradients). We used a three-channel, open-air circulation system to measure water loss (Figure 1d). Experimental design specifications, dimensions, and materials are available in SM2. Experiments lasted for 2.5 h. Once the data were extracted from data loggers, we obtained the minimum of relative humidity (RH) values from the five most stable periods of data recordings for further analysis, thus excluding the initial values of the experiment which tend to be the highest due to stress (Žagar et al., 2022). At the end of the fifth day, after water loss experiments, the lizards were fed with yellow mealworms and rested for another day. Lizards were then transported to a mesocosm where they were monitored during a week in natural conditions for habitat use and occupancy in a different study. Finally, the individually marked lizards were released at the exact collection points in Castro São Paio to avoid the recapture of individuals.

We evaluated water loss rates by estimating evaporative water loss (EWL) and effective proportion of surface area that is wet (p_{wet}) (Kearney et al., 2018; Kearney & Porter, 2017). A full description of the process to calculate EWL and p_{wet} is detailed in SM2. Subsequently, we performed ANOVA and ANCOVA models to test for differences in evaporative water loss and effective proportion of surface area that is wet between species, where we included body weight as a covariate, since body mass (rather than SVL) is an appropriate proxy for size in water loss analysis (Le Galliard et al., 2021). We also supported this decision based on the AIC values obtained, once we included body size or body weight as covariate (Table SM1.1). We also calculated the residual values of EWL and p_{wet} on weight (res. EWL and res. p_{wet}) for downstream multivariate analyses.

2.2.7 | Species position, overlap and association in multivariate phenotypic space

We performed a set of multivariate analyses to understand niche segregation between the two coexisting species in the multivariate phenotypic space composed by morphological, performance and physiological traits. To gain insights into the importance of body size for the segregation of niches of coexisting species and the contribution of different variables to phenotypic space once the effect of body size was removed, we performed these analyses twice: first

with the raw variables, that is including body size effects, and then with size-corrected variables. Before running multivariate analyses, and because our dataset included traits quantified in different scales and units, we standardized all variables by mean-centring them and scaling them to unit variance. To better explore phenotypic space and investigate which aspects of the phenotype are more relevant for niche segregation between species, we distinguished three variable subsets: one for morphology (MORPH), including trunk length, head size, hindlimb length and forelimb length; one for functional performance (PERFORM), including bite force, trunk max bending angle, head max bending angle, maximum sprint speed and maximum instantaneous speed in the double-L racetrack; and one for ecophysiology (ECOPHY), including mean preferred temperature, preferred temperature variance, evaporative water loss and proportion of surface area that is wet. The same subsets were considered using size-corrected variables (res.MORPH, res.PERFORM and res. ECOPHY). All analyses described below were therefore conducted considering a global dataset for the same individuals, with all traits together and each of the three subsets separately, both before and after size-correction.

To identify the most correlated phenotypic variables and the ones with the greatest weight in explaining their ordination in the multivariate space, we performed a principal component analysis (PCA) on the datasets. We used the 'factoextra' R package (Kassambara, 2016) to perform the PCA analysis and the prcomp function which uses a covariance matrix. Note, however, that since all variables were mean-centred and scaled before analyses, this is fully corresponding to an analysis using the correlation matrix. With this, we identified the amount of variation retained in each component and the associated variables. For the final representation of most of the datasets, we retained the axes that accounted for more than 50% of the variance. To test whether the multivariate phenotypic spaces composed of the subset datasets MORPH, PERFORM and ECOPHY differed significantly between species, and examine how each contributed to niche segregation, we performed a multivariate analysis of covariance (MANCOVA) considering size as a covariate and species as a factor, with the R package 'RRPP' (Collyer & Adams, 2019) and using residual randomization procedures with 1000 permutations.

To identify the phenotypic variables that maximize the separation between the two species we used discriminant analyses in all datasets. Due to the non-normality of some variables, we applied a flexible discriminant analysis (FDA), as implemented in the fda function of the 'mda' package (Hastie & Tibshirani, 2023). We extracted the linear discriminant scores and calculated their correlations with the predictor variables to identify the most important ones for species segregation. In addition, to investigate the multivariate association between the phenotypic spaces, we conducted a two-block partial least squares (PLS) regression with the plsr function of the R package pls (Mevik et al., 2011). We first used a PLS with raw variables in each set (MORPH, PERFORM, ECOPHY) and then we performed a PLS with size-corrected sets (res.MORPH, res. PERFORM, res. ECOPHY).

To obtain a comprehensive representation of the different phenotypic trait spaces defined by the subset datasets described above, and to evaluate niche overlap between species, we used a hypervolume analysis approach (Blonder, 2018; Blonder et al., 2014). Specifically, we calculated four hypervolumes, including those phenotypic trait combinations previously identified as most relevant for differentiating the two species. This resulted in a morphological hypervolume (including size-corrected hindlimb length, forelimb length and snout-to-vent length), a bite force hypervolume (size-corrected head size and bite force), a locomotor performance hypervolume (including size-corrected maximum instantaneous speed in the double-L racetrack, trunk max bending angle and head max bending angle) and an ecophysiological hypervolume (including size-corrected mean preferred temperature, preferred temperature variance and effective proportion of surface area that is wet). To estimate the shape and volume of multidimensional datasets and identify intersection (i.e. shared space that intersects between two or more hypervolumes), overlap and unique components within the hypervolumes, we used the 'hypervolume' package, and the functions `hypervolume`, `hypervolume_set`, `hypervolume_distance` and `hypervolume_overlap_statistics` (Blonder et al., 2018). For the construction of hypervolumes, we used the Silverman bandwidth estimator and the kernel density Gaussian method. To estimate the overlap of hypervolumes between species, we used the Sørensen and Jaccard indices, which are based on the similarity of the groups,

and range from 0 (no overlap) to 1 (complete overlap) (Blonder, 2018; Blonder et al., 2014).

3 | RESULTS

3.1 | Morphology

Timon lepidus and *Lacerta schreiberi* differed significantly in body size ($F=271.114$, $p=0.001$) and in all raw morphological traits due to body size (Table SM3.1), with *T. lepidus* being the largest species ($\bar{x}=144.3$ mm in *T. lepidus* vs. $\bar{x}=91.4$ mm in *L. schreiberi*, Table 1). However, we found no differences between species in trunk length and head size once the effect of body size was taken into account ($p>0.05$, Table SM3.1). On the other hand, forelimb length and hindlimb length showed significant differences between species even after accounting for size effects ($p<0.05$, Table SM3.1), with *T. lepidus* being the lizard with relatively longer limbs.

3.2 | Locomotor and bite performance

We did not identify significant differences in maximum sprint speed between the two species ($F=0.938$, $p=0.346$, Table 1). Likewise, we did not detect any relationship between sprint speed and body

TABLE 1 Mean values of variables obtained from morphological, performance and ecophysiological spaces.

Variable	<i>L. schreiberi</i>	<i>T. lepidus</i>	Significant differences	Significance adjusted for size
W	21.9 ± 4.3	84.5 ± 21.6	*	—
SVL	91.4 ± 7.4	144.3 ± 13.2	*	—
HS	19.7 ± 1.7	30.9 ± 3.5	*	—
TRL	46.1 ± 4.9	69.1 ± 7.3	*	—
HLL	45.7 ± 4.6	69.7 ± 5.2	*	*
FLL	32.4 ± 1.4	44.7 ± 3.1	*	*
BF	2.4 ± 0.1	2.8 ± 0.2	*	*
MaxSPR	5.6 ± 0.3	5.7 ± 0.4	—	—
Max_L	6.6 ± 2.9	5.2 ± 2.9	—	*
TA	147.1 ± 6.7	136.9 ± 12.2	*	—
HA	152.1 ± 10	151.4 ± 9.2	—	—
min_T	6.7 ± 2.9	5.15 ± 2.9	*	.
T_{pref}	30.4 ± 0.9	30.3 ± 0.6	—	—
$T_{\text{pref}} V$	4.5	2.4	*	—
EWL	0.9 ± 0.3	1.1 ± 0.4	*	—
p_wet	0.02 ± 0.008	0.01 ± 0.003	*	.

Note: The values are followed by the standard deviation of each variable. The abbreviations of the variables are as follows: Weight (W), snout to vent length (SVL), head size (HS), trunk length (TRL), fore limb length (FLL), hind limb length (HLL), bite force (BF), maximum sprint speed (MaxSPR), maximum trunk angle bending (TA), maximum head angle bending (HA), and minimum time to complete double-L racetrack (min_T), preferred temperature (T_{pref}), preferred temperature variance ($T_{\text{pref}} V$), evaporative water loss (EWL) and effective proportion of surface area that is wet (p_wet). Asterisks (*) denote statistically significant differences at an alpha level of 0.05 and dots (.) represent marginal differences ($p \approx 0.059$).

size or any of the other size-corrected morphological traits (e.g. size-corrected forelimb length and hindlimb length) nor their interaction with species ($p>0.05$ in all cases, [Table SM3.2](#)).

Concerning manoeuvrability, we found significant differences between species in trunk maximum bending angle ($F=9.394$, $p=0.004$), where *T. lepidus* exhibited lower angles than *L. schreiberi* [Table 1](#), but not in head maximum bending angle ($F=0.033$, $p=0.843$, [Table 1](#)). We found a significant negative relationship between trunk max bending angle and body size [Table 1](#), but once size effects were accounted for, differences between species were rendered non-significant ([Table SM3.2](#)). Variation in head max bending angle was not related to body size nor species ([Table SM3.2](#)). The maximum instantaneous speed in the double-L racetrack was not related to body size, and once size effects were considered, *L. schreiberi* was relatively faster ([Table SM3.2](#)). However, *L. schreiberi* took significantly longer to complete the racetrack ($F=3.898$, $p=0.048$, [Table 1](#)), and the time to complete the racetrack was inversely related to body size. Differences between species became marginally non-significant when accounting for size variation ([Table SM3.2](#)). Overall, *L. schreiberi* had a higher proportion of successful turns in the manoeuvrability experiments, and it showed a higher proportion of successful turns in the first turn ($\chi^2=6.232$, $p=0.012$, [Figure SM4.1](#)), but not in the second turn ($\chi^2=3\times 10^{-30}$, $p=0.999$, [Figure SM4.1](#)). *T. lepidus* had more crashes on the wall during the first turn ($\chi^2=5.552$, $p=0.018$, [Figure SM4.1](#)), but not during the second turn ($\chi^2=3\times 10^{-32}$, $p=0.999$, [Figure SM4.1](#)). *Lacerta schreiberi* was significantly more successful in stopping before both the first ($\chi^2=11.162$, $p=0.001$, [Figure SM4.1](#)) and the second turn ($\chi^2=9.267$, $p=0.002$, [Figure SM4.1](#)), whereas *T. lepidus* had a higher proportion of successful turns without stopping in the second ($\chi^2=15.121$, $p=0.0001$, [Figure SM4.1](#)), but not in the first turn ($\chi^2=2.275$, $p=0.131$, [Figure SM4.1](#)). Binomial mixed models showed that body size did not significantly relate to turning success, stops before the turn or maximum speed in the racetrack ($p>0.05$ in all cases, [Table SM3.3](#)). However, in the second turn, when maximum speed and stops before the turn were added to body size in the model, there was a significant effect on turn success and species ($p<0.05$, [Table SM3.3](#)).

There was also a significant positive relationship between bite force and body size, with *T. lepidus* exhibiting the highest raw bite force values ([Table 1](#)). Additionally, we detected a significant difference between species ([Table SM5.1](#)), with *L. schreiberi* showing relatively higher adjusted bite force values. However, we found no significant differences in bite force when using head size as a covariate, independent of body size ([Table SM5.1](#)).

3.3 | Preferred body temperatures and water loss rates

Mean and median preferred temperatures did not differ significantly between the two species ($F=0.066$, $p=0.840$ and $F=0.995$, $p=0.343$, respectively, [Table 1](#)). However, temperature variance

did ($F=21.347$, $p=0.001$): *L. schreiberi* showed greater amplitude in body temperature values and a skewed distribution towards lower temperatures than *T. lepidus* ([Figures SM5.1](#) and [SM5.2](#)). Body size had no effect on preferred temperature but significantly negatively affected temperature variance, making species differences non-significant ([Table SM5.2](#)). Raw evaporative water loss differed significantly between species ($F=6.731$, $p=0.001$), with *T. lepidus* showing higher values than *T. lepidus* ([Table 1](#)). However, this relationship became non-significant when accounting for body weight ([Table SM5.2](#)). The effective proportion of surface area that was wet was higher in *L. schreiberi* compared to *T. lepidus* ($F=126.12$, $p=0.001$, [Table 1](#)), but this difference became marginally non-significant when accounting for body weight ([Table SM5.2](#)).

3.4 | Species position, overlap and association in multivariate phenotypic space

PCA on the dataset of all raw variables showed two clearly distinguishable groups, corresponding to the two species ([Figure 2a](#)). The first two PCs retained about 56% of variation in the data ([Table SM6.1](#)). The most important variables for interpreting the PCs were the linear morphological variables, which were highly correlated, plus bite force for PC1; the ecophysiological variables (mean preferred temperature, preferred temperature variance, effective proportion of surface area that is wet and evaporative water loss); and the trunk maximum bending angle (TA) for PC2 ([Table SM6.2](#)). On the other hand, the PCA analysis with the size-corrected variables resulted in a high overlap between species ([Figure 2b](#)). The first two PCs retained 49% of variation ([Table SM6.3](#)). The size-corrected variables with the highest loadings on the PCs were size-corrected effective proportion of surface area that is wet, evaporative water loss, bite force and preferred temperature variance for PC1, and head size and trunk maximum bending angle for PC2 ([Table SM6.4](#)). Concerning the PCA analyses for raw morphological, performance and ecophysiological traits separately, the first two PCs captured 97%, 56%, and 64% of total variance, respectively ([Table SM6.5](#)). PC results for PERFORM and ECOPHY suggest that there was no major axis variation and rather indicate a spherical distribution of points in multivariate space ([Table SM6.5](#)). All variables were equally important for interpreting the PC1 in the MORPH dataset, and trunk length and hindlimb length for PC2; bite force and trunk maximum bending angle for PC1 and maximum sprint speed for PC2 in the PERFORM dataset; preferred temperature variance and evaporative water loss for PC1 and effective proportion of surface area that is wet for PC2 in the ECOPHY dataset ([Table SM6.6](#)). The relative overlap of PCs in MORPH variables showed two clearly distinguishable groups ([Figure 2c](#)), while in the PERFORM and ECOPHY datasets the PCs showed partial and total overlap respectively ([Figure 2e–g](#)). We identified that for the size-corrected res.MORPH, res.PERFORM and res.ECOPHY datasets the first two PCs cumulatively captured 62%, 56% and 75% of variance, respectively ([Table SM6.7](#)). The most important size-corrected variables for interpreting the PCs were size-corrected head size and

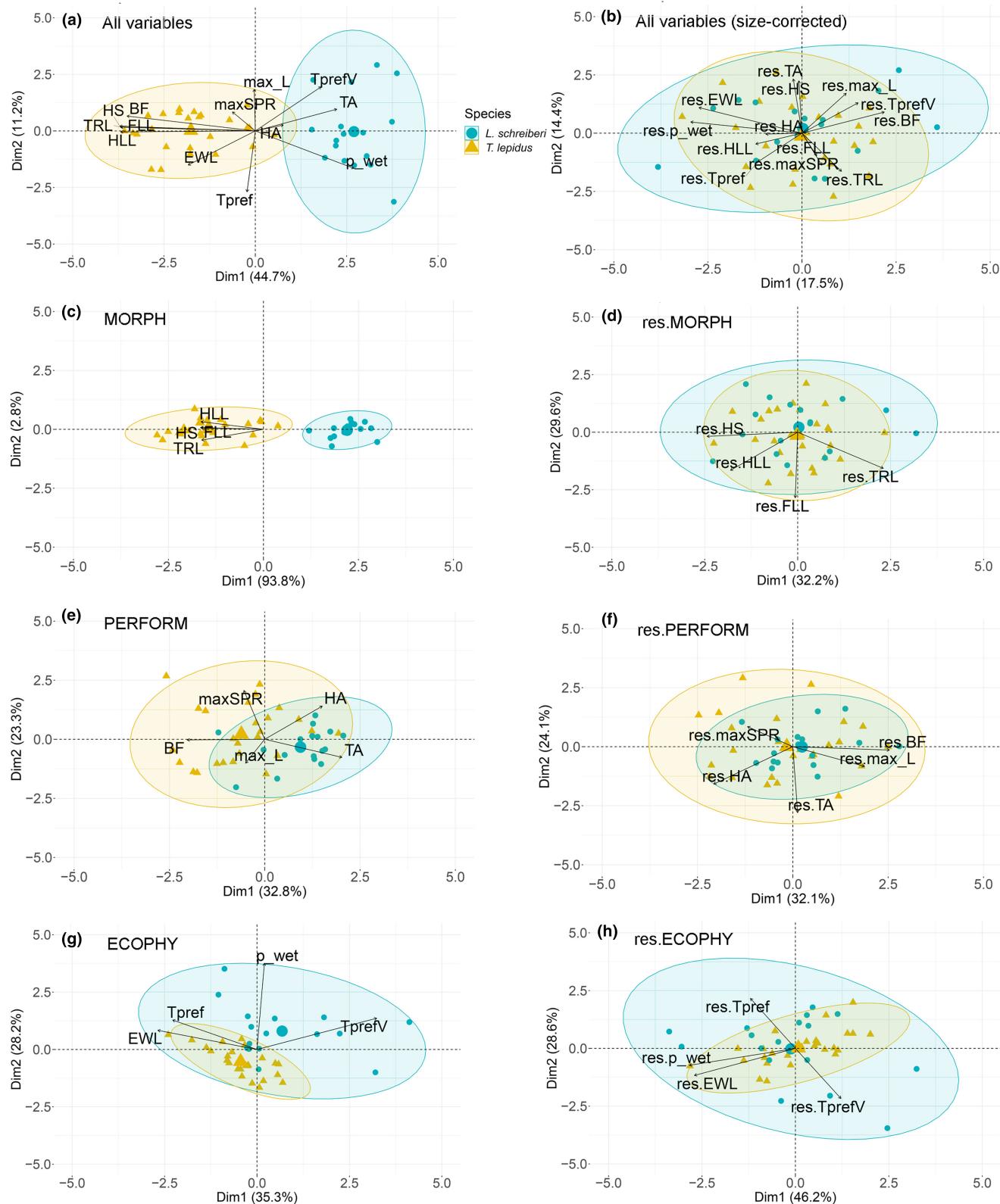


FIGURE 2 PCA plots representing the ordering of individuals of *Timon lepidus* (yellow triangles) and *Lacerta schreiberi* (blue dots) into the first two principal components of recorded phenotypic traits, before (left column) and after (right column) size correction, for different variable blocks. (a and b) All variables and (c and d) morphology (MORPH and res.MORPH datasets). (e and f) Functional performance (PERFORM and res.PERFORM datasets). (g and h) Ecophysiology (ECOPHY and res.ECOPHY datasets). The largest point and triangle in each group represents the centroid of each dataset.

trunk length for PC1 and forelimb length for PC2 in the res.MORPH dataset, bite force and head maximum bending angle for PC1 and trunk maximum bending angle for PC2 in the res.PERFORM, and effective proportion of surface area that is wet and evaporative water loss for PC1 and mean preferred temperature and preferred temperature variance for PC2 in the res.ECOPHY (Table SM6.8). Within the size-corrected datasets, we identified that trunk length (res.TRL) was inversely related to head size, forelimb length and hindlimb length (res.HS, res.FLL and res.HLL). Likewise, bite force was inversely related to the components of manoeuvrability and sprint speed (res.HA, res.TA and res.MaxSRP). On the other hand, evaporative water loss (res.EWL) and surface area that is wet (res.p_wet) were positively correlated, while preferred temperature variance (res.TprefV) and preferred temperature (Tpref) were inversely related among them (Table SM6.8; Figure 2h). The relative overlap of PCs was high in res.MORPH, res.PERFORM and res.ECOPHY (Figure 2).

We found that, without correcting for body size effects, the variables with the greatest weight for discriminating species included all morphological traits, as well as bite force and to a lesser extent evaporative water loss, effective proportion of surface area that is wet, trunk maximum bending angle and preferred temperature variance (Figure 3; Table SM6.9). Once size effects were removed from the data, the variables that showed the greatest importance for species discrimination included forelimb length and maximum sprint speed in the positive and mean preferred temperature and bite force in the negative direction (Figure 3c; Table SM6.9).

When we separated the raw variables MORPH, PERFORM and ECOPHY datasets, we detected that morphological variables are

equally important with slightly greater weight on limb length for the separation between groups (Figure 3b; Table SM6.9) in the MORPH dataset, bite force in the positive and trunk maximum bending angle in the negative direction (Figure 3c; Table SM6.9) in the PERFORM dataset, and evaporative water loss with positive direction, effective proportion of surface area that is wet in a negative direction (Figure 3d; Table SM6.9) in the ECOPHY dataset. When we separated variables obtained from their residuals (size-corrected), forelimb length, hindlimb length and head maximum bending angle, and bite force were the variables with the highest weight in res.MORPH and res.PERFORM dataset, respectively (Figure 3; Table SM6.9). By contrast, in the res.ECOPHY dataset the variables with the highest weight were the size-corrected preferred temperature variance and effective proportion of surface area that is wet (Figure 3; Table SM6.9). The two-block partial least-squares regression performed on raw variables showed significant association between MORPH and PERFORM ($r=0.85, p<0.01$), ECOPHY and MORPH ($r=0.85, p<0.01$) and also between PERFORM and ECOPHY ($r=0.77, p<0.01$). On the other hand, in the size-corrected data the PLS still showed significant association between res.MORPH and res.PERFORM ($r=0.49, p<0.01$), res.PERFORM and res.ECOPHY ($r=0.33, p\leq 0.05$). PLS showed no association between res.MORPH and res.ECOPHY ($r=0.02, p=0.87$). Details on PLSs results can be found on SM7.

The MANCOVA analysis showed a significant effect of body size when considering all raw variables (Table 2). However, we found no differences between species. The MANCOVA analysis for each dataset showed significant differences between species for the MORPH

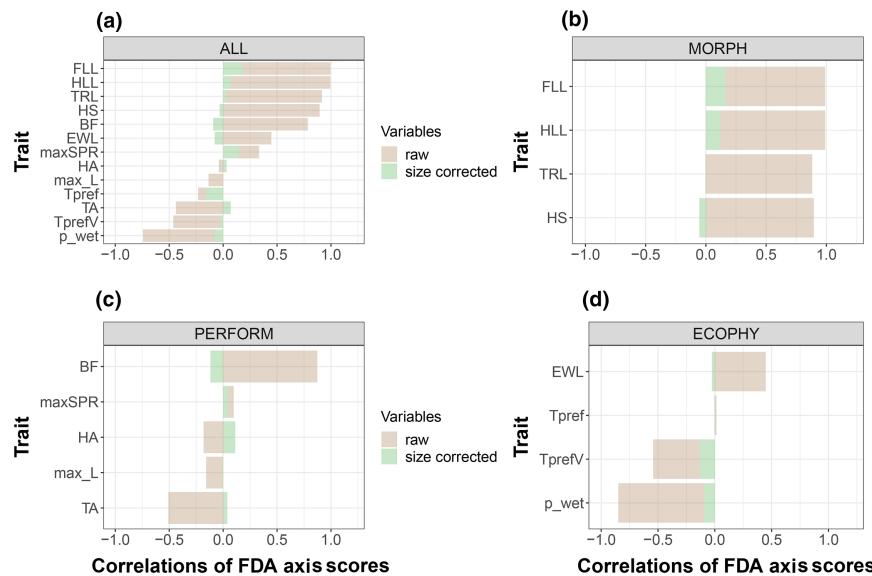


FIGURE 3 Correlations of FDA axis scores with phenotypic variables in two species of green lizards (*Timon lepidus* and *Lacerta schreiberi*). (a) FDA axes scores considering all raw and size-corrected variables in the dataset together. (b) FDA axes scores considering MORPH raw and size-corrected variables. (c) FDA axes scores considering PERFORM raw and size-corrected variables. (d) FDA axes scores considering ECOPHY raw and size-corrected variables (MORPH, PERFORM, ECOPHY). The abbreviations of the variables are as follows: Trunk length (TRL), head size (HS), fore limb length (FLL), hind limb length (HLL), maximum sprint speed (MaxSPR), maximum speed in the double-L racetrack (Max_L), maximum trunk angle bending (TA), maximum head angle bending (HA), bite force (BF), preferred temperature (T_{pref}), preferred temperature variance ($T_{pref}V$), evaporative water loss (EWL) and effective proportion of surface area that is wet (p_wet).

and ECOPHY datasets even when the effect of body size was taken into account (Table 2). In contrast, the PERFORM dataset exhibited no differences between species (Table 2).

Complementarily, we quantitatively identify multivariate spaces using a hypervolume approach. Hypervolume overlap analyses

TABLE 2 MANCOVA table reporting multivariate comparisons between species in different datasets, considering body size as a covariate.

MANCOVA	df	Roy	Z	Pr(>Roy)
All variables				
Size	1	19.195	12.189	0.001
Species	1	0.056	0.401	0.356
Size × Species	1	0.055	0.393	0.359
Full model	3	19.249	11.429	0.001
Residuals	39			
MORPH				
Size	1	39.766	13.136	0.001
Species	1	0.228	1.961	0.023
Size × Species	1	0.032	-0.122	0.552
Full model	3	39.959	14.602	0.001
Residuals	39			
PERFORM				
Size	1	1.140	4.520	0.001
Species	1	0.090	0.944	0.188
Size × Species	1	0.028	-0.257	0.604
Full model	3	1.153	4.069	0.001
Residuals	39			
ECOPHY				
Size	1	1.652	5.651	0.001
Species	1	0.176	1.687	0.044
Size × Species	1	0.090	0.823	0.218
Full model	3	1.889	4.992	0.001
Residuals	39			

Note: Variables' abbreviations are shown in the methodology section. *p* value in bold when alpha $\alpha < 0.05$.

Abbreviations: df, degrees of freedom; Roy, Roy's maximum root.

showed that hypervolumes associated with bite force and performance had the greatest overlap, whereas morphological and ecophysiological hypervolumes had the least overlap (Table 3); however, the morphological hypervolume was the only one that included body size, and its difference in overlap was mainly due to the inclusion of this variable (Figure 4). The degree of hypervolume intersection varied between the morphological ($\approx 1.7\%$; due to body size; see Figure 4), ecophysiological ($\approx 16\%$), performance ($\approx 64\%$) and bite force hypervolumes ($\approx 74\%$) (Table 3). Hypervolume overlap assessed through Sørensen and Jaccard similarity indices showed that the morphological hypervolume including body size has the lowest degree of overlap between species, while the performance hypervolumes (locomotor traits and bite force) have the highest degree of overlap. On the other hand, the ecophysiological hypervolumes showed that even when body size was removed, there was separation of spaces between species (Table 3).

4 | DISCUSSION

The phenotypic space is multidimensional, including all traits associated with morphology, physiology and behaviour (Murren, 2012; Wojczynski & Tiwari, 2008).

With different ecological functions tightly linked to each of the axes of this multidimensional phenotypic space, niche segregation along some or all of those axes can facilitate species coexistence (Valladares et al., 2015). However, research combining several phenotypic dimensions to systematically understand species coexistence and the role of size variation on different aspects of the phenotype are scarce. To address this research gap, we performed a comprehensive comparison of different phenotypic dimensions and their degree of overlap/segregation in two coexisting lizard species, while also putting body size variation into perspective.

By integrating the multivariate phenotypic space including morphological, functional performance and ecophysiological traits we were able to identify body size as the main driver that dominates variation, and therefore a major factor contributing to niche segregation. This reinforces the importance of size, which mediates most of the functions of organisms from metabolism (Angilletta Jr., 2009)

TABLE 3 Metrics of phenotypic space occupancy and overlap estimated through hypervolume analyses between the two species of green lizards from northern Portugal.

Space	Species	Volume	Intersection	% intersection	Unique	% unique	Jaccard	Sørensen
Morphological	<i>T. lepidus</i>	0.024	0.008	1.70	0.022	97.02	0.015	0.0284
	<i>L. schreiberi</i>	0.035			0.017	96.17		
Bite force	<i>T. lepidus</i>	20.853	18.325	74.03	2.529	12.13	0.741	0.851
	<i>L. schreiberi</i>	22.225			3.900	17.55		
Locomotor performance	<i>T. lepidus</i>	95.965	76.147	63.73	19.818	20.65	0.637	0.779
	<i>L. schreiberi</i>	99.656			23.510	23.59		
Ecophysiology	<i>T. lepidus</i>	15.889	3.193	15.75	12.695	79.90	0.154	0.267
	<i>L. schreiberi</i>	7.571			4.378	57.83		

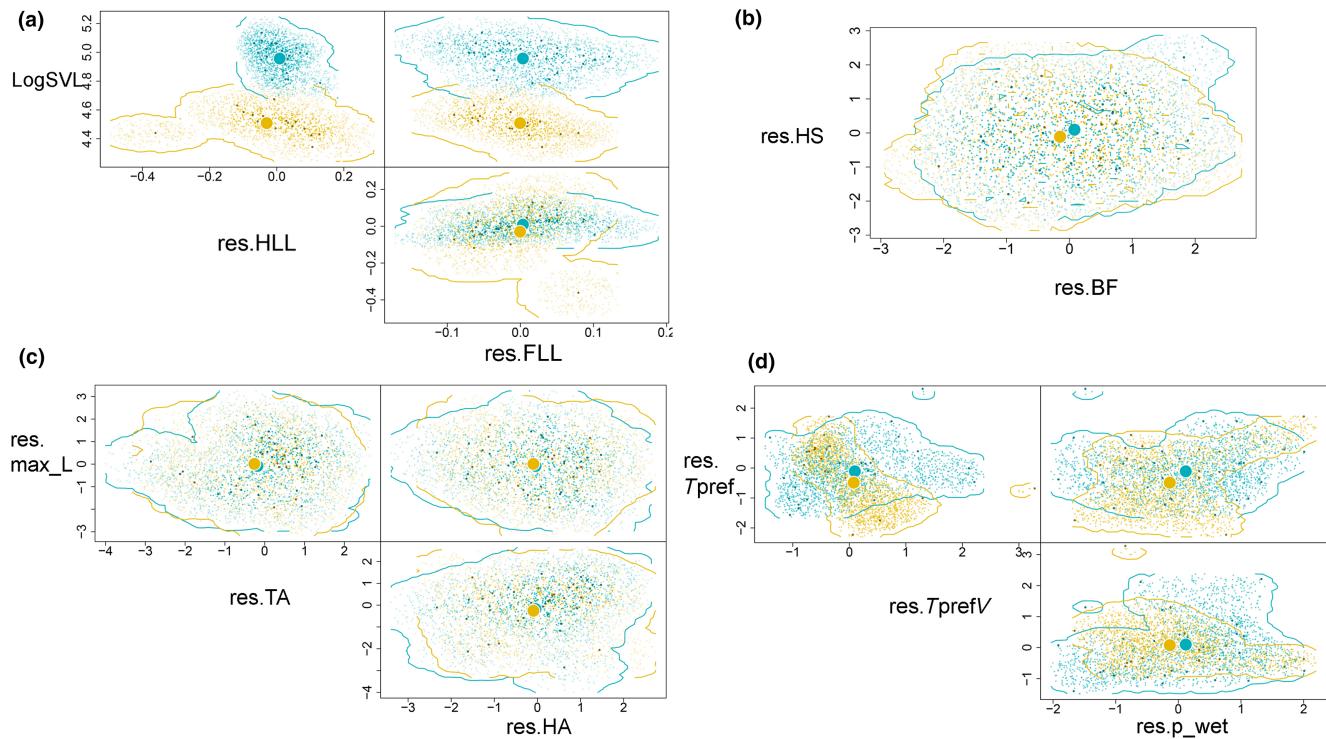


FIGURE 4 Phenotypic hypervolumes of *Timon lepidus* (yellow) and *Lacerta schreiberi* (blue). (a) Morphological hypervolume, (b) bite force hypervolume, (c) locomotor performance hypervolume and (d) ecophysiological hypervolume. Small dots represent resampled points derived from original data values while big dots represent the hypervolume centroids. The abbreviations of the variables are as follows: Snout to vent length (SVL), size-corrected head size (res.HS), size-corrected fore limb length (res.FLL), size-corrected hind limb length (res.HLL), size-corrected maximum speed in the double-L racetrack (res.max_L), maximum trunk angle bending corrected by size (res.TA), head angle bending corrected by size (res.HA), size-corrected bite force (res.BF), preferred temperature corrected by size (res. T_{pref}), preferred temperature variance corrected by size (res. $T_{\text{pref}}V$) and effective proportion of surface area that is wet corrected by size (res. p_{wet}).

to reproductive and behavioural strategies (Eldøy et al., 2021; Quinn & Foote, 1994; Wells, 1988). The effect of body size on phenotypic traits is pervasive, causing most other traits to respond directly to body size scaling (Chown & Gaston, 2010; Chown & Klok, 2003; Laube et al., 2013). Size effects can directly impact the trophic niche, for example, where larger animals with larger heads consume larger prey items that are not available to smaller organisms (Kaliontzopoulou et al., 2012). Instead, by looking at size-free phenotypic spaces it is possible to unveil the extent to which specific traits further contribute to niche segregation in co-occurring species, independently of body size. Across the three distinct phenotypic spaces, we detected that only the morphological and ecophysiological spaces maintain niche segregation after body size effects were accounted for. Specific traits in each space appear to have a greater contribution to such segregation, including relative limb length and traits associated with both thermal and hydric physiology. Other investigations have been concordant in finding size-free axes of major importance for niche segregation (Attum et al., 2007; Lu et al., 2011; Redtfeldt & Davis, 1996). For instance, in studies with Darwin's finches it has been observed that beak size, independent of body size, correlates with the ability to exploit different types of seeds, influencing diet preferences and ecological niches in coexisting species (Herrel et al., 2005; Van der Meij & Bout, 2006). Another example

comes from dune lizards, where changes in morphological traits such as limb lengths and their relationship to habitat adaptations, highlighting the importance of size-independent traits in understanding habitat preferences and strategies for coping with coexistence (Attum et al., 2007). Although several authors have recently emphasized the relevance of understanding multivariate niche spaces (e.g. Anderson et al., 2023; Carvalho & Cardoso, 2020; Castro-Arellano & Lacher, 2009), studies that implement an integrated framework to disentangle the different axes of phenotypic space and better understand the mechanisms that contribute to species coexistence are limited.

For instance, the interplay between performance and morphology is a well-known established paradigm for understanding phenotypic trait coevolution (Arnold, 1983). Here, it aids us in gaining a clearer understanding on how segregation occurs to support species coexistence. It appears that, at least in the system of green lizards studied here, body size contributes to species differentiation in traits related to trophic ecology and spatial behaviour, such as bite force and angles of locomotor manoeuvrability (Gomes et al., 2018; Huyghe et al., 2005; Vasilopoulou-Kampitsi, 2020; Verwijnen et al., 2002), but not in other traits such as maximum speed (Gomes et al., 2016; Irschick & Losos, 1998). Interestingly, other studies have already reported sprint speed equivalence in

co-existing species (Gomes et al., 2018; Žagar et al., 2017), and apparently the lack of direct competition between *T. lepidus* and *L. schreiberi* could be related to our results. Furthermore, *T. lepidus* uses more open spaces, in which it might encounter fewer obstacles when escaping, while *L. schreiberi* is more associated with vertical vegetation (Salvador, 1988), so it probably manoeuvres better in this stratum. Further differences between species existed in the strategy used for successfully manoeuvring and turning in complex environments, where *L. schreiberi* turned successfully when stopping before curving at the double L-racetrack angles, while, on the contrary, *T. lepidus* tended not to stop before curving. Put together, these results suggest that predator escape or territory defence, as might be reflected in sprinting performance, are not as important for niche segregation, but instead the conformation of the structural habitat used by both species seems more relevant. Indeed, *L. schreiberi* typically moves within and on top of vegetation and strata such as shrubs, ferns, etc., both for thermoregulation and when escaping predators, and it even has the ability to swim (Brito, Paulo, & Crespo, 1998; Brito, Luis, et al., 1998; Mateo, 2017). This kind of spatial behaviour gives it a potential advantage of first observing the environment and then, in case of an eventual threat, deciding to flee. Instead, *T. lepidus* tends to move in more open and exposed environments, and it typically has a fast escape behaviour mainly on the ground or on rock walls (Fargallo et al., 2020; Mateo, 2017; Sannolo et al., 2019). Thus, the behaviour of organisms affects their performance and other phenotypic traits of the niche. Some individuals depending on their size may be bolder to run or may have different strategies to cope with species' interactions (e.g. more exploratory behaviour) (Damas-Moreira et al., 2019; Žagar et al., 2022). Thus, in the context of species coexistence, mechanisms to segregate niches may also relate to complex behavioural strategies and trophic level (e.g. size-dependent bite force) (Herrel et al., 2001; Ho et al., 2021). Preliminary results with the individuals analysed in this work using stable isotopes have shown that *T. lepidus* and *L. schreiberi* differ in their overall diet based on the proportions of carbon and nitrogen signatures (unpublished data).

In the context of our study and with the aim of understanding niche segregation free from the effect of body size in coexisting species, we followed a multivariate strategy. From this perspective, the size-corrected morphological space examined in isolation shows differentiation between species (Table 2). The results of the PCA, FDA, and univariate data (Table SM3.1) establish relative limb lengths as the main traits contributing to species differentiation in the morphological space, after accounting for variations in body size. However, we did not find a direct relationship with any of the variables studied in our experiments. Limb variation may be a consequence of differential habitat use that is not related to escape speed in our study models. As mentioned by other authors (Brito, Paulo, & Crespo, 1998; Marco & Pollo, 1993) and by our direct observations in Castro de São Paio, *T. lepidus* uses more open areas, and *L. schreiberi* tends to occupy more closed and shrubby areas. So, these variations in the composition of the vertical stratum may shape the morphology of the limbs, where lizards that use open areas tend to

change their limb posture and vary the use of the habitat stratum (Fuller et al., 2011; Vanhooydonck & Van Damme, 2003).

On the other hand, species distribution in (size corrected) ecophysiological space highlights that, apart from the metabolic functions that respond directly to body size (Angilletta Jr., 2009; Angilletta Jr et al., 2004; Nagy, 2005), there are size-free processes that differentiate species and may therefore contribute to a more efficient segregation of the ecophysiological niche. A closer examination of individual ecophysiological traits revealed that mean and median preferred temperature was not different between species, but the temperature variance was, and it was also negatively related to body size. That is, smaller individuals showed a greater temperature variance during the experimental period, with greater variance in the afternoon hours in contrast to the morning hours (Figure SM5.2). This variation may be due to constraints associated with morphological traits, thermoregulation behaviour, metabolic rate variation or genetic variability (Angilletta Jr., 2009; Kohlsdorf & Navas, 2006; Phillips et al., 2016; Sannolo et al., 2018; S'khifa et al., 2020). Thus, one of the likely explanations for this variation in preferred temperatures is that the thermoregulation of *L. schreiberi* may be more limited by its hydro-regulatory processes. As such, *L. schreiberi* may struggle to maintain its T_{pref} over longer periods of time, unlike *T. lepidus*. Consequently, individuals have more variable body temperatures leading to an overall skewed distribution of temperatures (as shown in Figure SM6.1). In this context larger animals tend to retain heat more effectively and for a longer period of time (thermal inertia), while smaller animals lose heat more easily (Angilletta Jr., 2009), which could change their thermoregulatory behaviour and increase the variance in temperature. Concerning water loss, it can be explained primarily by differences in skin exposure relative to body size. However, adaptations associated with skin texture and morphology may influence the rates associated with evaporative water loss (Eynan & Dmi'el, 1993) even when the effect of body size is removed. Although size-corrected evaporative water loss was not different between the two species (Table SM6.2) and the effective surface area that is wet was marginally non-significant (Table SM6.2), the multivariate results from the ECOPHY dataset (Tables 2 and 3; Figure 4) reinforce that this space is highly relevant for species segregation, when variables are considered in concert. These results make sense in light of field observations at a geographic scale, where *L. schreiberi* has a more hydrophilic behaviour, while *T. lepidus* is more related to the Mediterranean climate (Llorente et al., 1995).

The partitioning of multivariate spaces allows us to understand that niche segregation occurs simultaneously on several combined axes of the phenotype, rather than on univariate traits. For example, univariate ecophysiological traits (i.e. mean preferred temperature, preferred temperature variance, effective surface area that is wet) did not show clear differences between species. However, when we used a multivariate approach and assessed the entire ecophysiological phenotypic space, we verified that species' segregation is evident (Tables 2 and 3). Our field observations on thermal and hydric ecology, conducted separately, align closely

with those from our laboratory results (pers. obs.). Additionally, our observations suggest microhabitat differences in relative humidity between the two species, alongside temporal activity variations, which may contribute to niche segregation, as has already been observed in other organisms (Martínez-Freiría et al., 2010; Navarro et al., 2013).

Contrary to our expectations, we detected greater intersection and therefore suggested overlap of the corresponding niches in the size-corrected hypervolumes of variables associated to functional performance (bite force and locomotor performance). Indeed, the shape of these hypervolumes was homogeneous and without gaps between species (Figure 4b,c). On the other hand, the hypervolume representing the ecophysiological niche showed gaps and shape constrictions in the niche spaces (Figure 4d). By contrast, species segregation across the morphological hypervolume was totally dominated by body size, as we had predicted (Figure 4a). However, the phenotypic space associated with relative limb lengths still encompassed distinct hypervolumes for each species (Figure 4a). Interestingly, these results suggest that while body size facilitates niche segregation mostly through phenotypic shifts (changes in average position across phenotypic space), the ecophysiological space displays differentiation mainly through niche expansions and contractions (differences in niche amplitude) (Carvalho & Cardoso, 2020). Niche expansions refer to the broadening of a species' ecological niche, allowing it to tolerate a wider range of ecological components. On the other hand, niche contractions imply a reduction of the niche, indicating a more specialized and limited tolerance to particular ecological factors (Carvalho & Cardoso, 2020). In general terms, these species overlap their geographic distribution in certain regions, mainly in the north of the Iberian Peninsula. However, the complex spatial arrangement could provide niche spaces (e.g. ecophysiology, behaviour) for both species, which seem mainly when considering spaces related to water ecology, as thermal ecology seems to be more phylogenetically restricted within the family (Enriquez-Urzelai et al., 2022; Garcia-Porta et al., 2019; S'khifa et al., 2022). In this sense, multivariate phenotypic spaces are complex, and their interpretation becomes easier when decomposing them into variables that represent different niche aspects (Carvalho & Cardoso, 2020; Holt, 2009; Hothorn et al., 2011). By analysing our variables in separate hypervolumes, we were able to better understand each space, with a restricted number of variables responding to a particular dimension and to evaluate their relative contribution towards coexisting species' segregation.

Our findings confirm the usefulness of establishing integrated multivariate spaces not only to better understand ecological niche characterization but also niche segregation in coexisting species. This methodological view incorporating different lines of evidence is key to elucidate the mechanisms that facilitate coexistence among species. Taking into account our experimental trait view, we show that the ecological niche can be effectively segregated even when body size is removed from the phenotypic space. Trait-based approaches have been effective in integrating functional ecology with

species coexistence theories, considering that trait dissimilarity is linked to reduced niche overlap between coexisting species (Kraft et al., 2008; Stubbs & Bastow Wilson, 2004). The view of species coexistence is not merely the competition for resources but also involves complex ecological interactions shaped by factors that define the niche (McPeek, 2014). This is confirmed by our results, in which physiological traits act differently in each species, forming a segregated ecological niche. When species are similar in size, direct interspecific competition with interference may be expected (Martin & Martin, 2001; Zeng & Lu, 2009). However, when body sizes are relatively different, and trophic resources are available there are other areas of the niche that can be exploited (i.e. use of microhabitat strata, dietary composition), for example, size allows species to lengthen or shorten the dietary profile (Damas-Moreira et al., 2020; Lu et al., 2011; Gomez et al., unpublished data) without competing directly, although exploitative competition via ecological resources remains possible. Ecophysiological space has been shown in other research to be useful for understanding the mechanisms by which niche segregation may be enhanced (Horn & Riegler, 1981; Le Lagadec et al., 1998). With our contribution, we highlight that through niche segregation of ecophysiological traits, aided by a less marked segregation in other aspects of performance, organisms are able to exploit the available resources. This culminates in a differential use of the structural niche (free of size effect), including its microclimatic and physical characteristics, thus allowing species to potentially reduce direct competition.

AUTHOR CONTRIBUTIONS

Carolina Reyes-Puig conceived the ideas and designed the methodology, collected the data, analysed the data and led the writing of the manuscript. Urtiz Enriquez-Urzelai conceived the ideas and designed the methodology, collected the data, analysed the data and actively participated in the revision of the manuscript. Miguel A. Carretero designed the methodology, analysed the data and actively participated in the revision of the manuscript. Antigoni Kaliontzopoulou conceived the ideas and designed the methodology, collected the data, analysed the data and actively participated in the revision of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We are extremely grateful to Katerina Sioumpoura, Frederico Barroso, Pablo Vincent, Verónica Gomes, Neftalí Sillero, Duarte Oliveira, David Brito-Zapata, Gorki Ríos-Alvear and Ankalli Ríos-Reyes who assisted at different stages of the laboratory experiments.

FUNDING INFORMATION

This work was supported by FEDER Funds through the Operational Competitiveness Factors Program—COMPETE and National Funds through FCT—Foundation for Science and Technology within the scope of the projects 'PTDC/BIA-EVL/28090/2017-POCI-01-0145-FEDER-028090' and 'PTDC/BIA-CBI/28014/2017-POCI-01-0145-FEDER-028090'. AK is supported by a Ramón

y Cajal research grant co-funded by the Spanish State Research Agency and the European Social Fund (RYC2019-026688-I/AEI/10.13039/501100011033). This work was supported by Universidad San Francisco de Quito, Museo de Zoología & Laboratorio de Zoología Terrestre, Instituto de Biodiversidad Tropical IBIOTROP granted to CRP (project HUBI 17162). CRP expressly thanks Russell E. Train Education for Nature Initiative from WWF for the support for the development of this research, and Asociación Española de Ecología Terrestre (AEET) for providing important funds through the call 'Tomando la Iniciativa, 2021'.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The files necessary to reproduce data analyses are available in the following Zenodo Digital Repository: <https://doi.org/10.5281/zenodo.13345226>.

ORCID

Carolina Reyes-Puig  <https://orcid.org/0000-0001-7828-8698>
 Urtzi Enríquez-Urzelai  <https://orcid.org/0000-0001-5958-2250>
 Miguel A. Carretero  <https://orcid.org/0000-0002-2335-7198>
 Antigoni Kalontzopoulou  <https://orcid.org/0000-0002-7897-7204>

REFERENCES

- Aasa, U., Jaric, S., Barnekow-Bergkvist, M., & Johansson, H. (2003). Muscle strength assessment from functional performance tests: Role of body size. *The Journal of Strength & Conditioning Research*, 17(4), 664–670. [https://doi.org/10.1519/1533-4287\(2003\)017<0664:msaffp>2.0.co;2](https://doi.org/10.1519/1533-4287(2003)017<0664:msaffp>2.0.co;2)
- Amarasekare, P. (2002). Interference competition and species coexistence. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269(1509), 2541–2550. <https://doi.org/10.1098/rspb.2002.2181>
- Anderson, R. O., Tingley, R., Hoskin, C. J., White, C. R., & Chapple, D. G. (2023). Linking physiology and climate to infer species distributions in Australian skinks. *Journal of Animal Ecology*, 92(10), 2094–2108. <https://doi.org/10.1111/1365-2656.14000>
- Angilletta, M. J., Jr., Steury, T. D., & Sears, M. W. (2004). Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. *Integrative and Comparative Biology*, 44(6), 498–509. <https://doi.org/10.1093/icb/44.6.498>
- Angilletta, M. J., Jr. (2009). *Thermal adaptation: A theoretical and empirical synthesis*. Oxford University Press.
- Armstrong, R. A., & McGehee, R. (1976). Coexistence of species competing for shared resources. *Theoretical Population Biology*, 9(3), 317–328. [https://doi.org/10.1016/0040-5809\(76\)90051-4](https://doi.org/10.1016/0040-5809(76)90051-4)
- Arnold, S. J. (1983). Morphology, performance and fitness. *American Zoologist*, 23, 347–361.
- Attum, O., Eason, P., & Cobbs, G. (2007). Morphology, niche segregation, and escape tactics in a sand dune lizard community. *Journal of Arid Environments*, 68(4), 564–573. <https://doi.org/10.1016/j.jaridenv.2006.07.010>
- Ayers, D. Y., & Shine, R. (1997). Thermal influences on foraging ability: Body size, posture and cooling rate of an ambush predator, the python *Morelia spilota*. *Functional Ecology*, 11(3), 342–347. <https://doi.org/10.1046/j.1365-2435.1997.00093.x>
- Barroso, F. M., Riaño, G., Sannolo, M., Carretero, M. A., & Rato, C. (2020). Evidence from *Tarentola mauritanica* (Gekkota: Phyllodactylidae) helps validate thermography as a tool to infer internal body temperatures of lizards. *Journal of Thermal Biology*, 93, 102700. <https://doi.org/10.1016/j.jtherbio.2020.102700>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *arXiv preprint*, arXiv:1406.5823. <https://doi.org/10.18637/jss.v067.i01>
- Baudier, K., & O'Donnell, S. (2018). Complex body size differences in thermal tolerance among army ant workers (*Ecton burchellii parvispinum*). *Journal of Thermal Biology*, 78, 277–280. <https://doi.org/10.1016/j.jtherbio.2018.10.011>
- Bedriaga, J. (1879). Herpetologische Studien. *Archiv für Naturgeschichte*, XLIV, 259–320.
- Blonder, B. (2018). Hypervolume concepts in niche-and trait-based ecology. *Ecography*, 41(9), 1441–1455. <https://doi.org/10.1111/ecog.03187>
- Blonder, B., Lamanna, C., Viole, C., & Enquist, B. J. (2014). The n-dimensional hypervolume. *Global Ecology and Biogeography*, 23(5), 595–609. <https://doi.org/10.1111/geb.12146>
- Blonder, B., Morrow, C. B., Maitner, B., Harris, D. J., Lamanna, C., Viole, C., Enquist, B., & Kerkhoff, A. J. (2018). New approaches for delineating n-dimensional hypervolumes. *Methods in Ecology and Evolution*, 9(2), 305–319. <https://doi.org/10.1111/2041-210X.12865>
- Brito, J. C., Luís, C., Godinho, M. R., Paulo, O. S., & Crespo, E. G. (1998). Bases para a Conservação do Lagarto-de-água (*Lacerta schreiberi*). *Estudos de Biologia e Conservação da Natureza no 23 Instituto da Conservação da Natureza Ministério do Ambiente, Lisboa*.
- Brito, J. C., Paulo, O. S., & Crespo, E. G. (1998). Distribution and habitats of Schreiber's green lizard (*Lacerta schreiberi*) in Portugal. *Herpetological Journal*, 8(4), 187–194.
- Carlson, S. M., Olsen, E. M., & Vøllestad, L. A. (2008). Seasonal mortality and the effect of body size: A review and an empirical test using individual data on brown trout. *Functional Ecology*, 22, 663–673. <https://doi.org/10.1111/j.1365-2435.2008.01416.x>
- Carretero, M. A., Roig, J. M., & Llorente, G. A. (2005). Variation in preferred body temperature in an oviparous population of *Lacerta (Zootoca) vivipara*. *The Herpetological Journal*, 15(1), 51–55.
- Carvalho, J. C., & Cardoso, P. (2020). Decomposing the causes for niche differentiation between species using hypervolumes. *Frontiers in Ecology and Evolution*, 8, 243. <https://doi.org/10.3389/fevo.2020.00243>
- Castro-Arellano, I., & Lacher, T. E. (2009). Temporal niche segregation in two rodent assemblages of subtropical Mexico. *Journal of Tropical Ecology*, 25(6), 593–603. <https://doi.org/10.1017/S026646740990186>
- Chown, S. L., & Gaston, K. J. (2010). Body size variation in insects: A macroecological perspective. *Biological Reviews*, 85(1), 139–169. <https://doi.org/10.1111/j.1469-185X.2009.00097.x>
- Chown, S. L., & Klok, C. J. (2003). Water-balance characteristics respond to changes in body size in subantarctic weevils. *Physiological and Biochemical Zoology*, 76(5), 634–643. <https://doi.org/10.1086/376919>
- Collyer, M. L., & Adams, D. C. (2019). RRPP: Linear model evaluation with randomized residuals in a permutation. Procedure. <https://CRAN.R-project.org/package=RRPP>
- Colwell, R. K., & Rangel, T. F. (2009). Hutchinson's duality: The once and future niche. *Proceedings of the National Academy of Sciences of the United States of America*, 106(supplement_2), 19651–19658. <https://doi.org/10.1073/pnas.0901650106>
- Damas-Moreira, I., Riley, J. L., Carretero, M. A., Harris, D. J., & Whiting, M. J. (2020). Getting ahead: Exploitative competition by an invasive lizard. *Behavioral Ecology and Sociobiology*, 74, 117. <https://doi.org/10.1007/s00265-020-02893-2>
- Damas-Moreira, I., Riley, J. L., Harris, D. J., & Whiting, M. J. (2019). Can behaviour explain invasion success? A comparison between

- sympatric invasive and native lizards. *Animal Behaviour*, 151, 195–202. <https://doi.org/10.1016/j.anbehav.2019.03.008>
- Daudin, F. M. (1802). *Histoire Naturelle, générale et particulière des reptiles, ouvrage faisant suite, à l'histoire naturelle, générale et particulière composée par Leclerc de Buffon, et redigée par C. S. Sonnini*. *Dufart*, 3, 452.
- de la Vega, G. J., & Schilman, P. E. (2017). Using eco-physiological traits to understand the realized niche: The role of desiccation tolerance in Chagas disease vectors. *Oecologia*, 185(4), 607–618. <https://doi.org/10.1007/s00442-017-3986-1>
- Delgado, J. D., & Gómez, M. D. L. Á. (2016). Evidence of use of road drainage culverts by *Timon lepidus* (Daudin, 1802) in Western Andalusia. *Anales de Biología*, 38, 63–67. <https://doi.org/10.6018/analesbio.38.05>
- Eldøy, S. H., Bordeleau, X., Lawrence, M. J., Thorstad, E. B., Finstad, A. G., Whoriskey, F. G., Crossin, G. T., Cooke, S. J., Aarestrup, K., Rønning, L., Sjursen, A. D., & Davidsen, J. G. (2021). The effects of nutritional state, sex and body size on the marine migration behaviour of sea trout. *Marine Ecology Progress Series*, 665, 185–200. <https://doi.org/10.3354/meps13670>
- Elton, C. (1927). The animal community. In C. S. Elton (Ed.), *Animal ecology* (pp. 239–256). The Macmillan Company.
- Enriquez-Urzelai, U., Martínez-Freiría, F., Freitas, I., Perera, A., Martínez-Solano, I., Salvi, D., Velo-Antón, G., & Kaliontzopoulou, A. (2022). Allopatric speciation, niche conservatism and gradual phenotypic change in the evolution of European green lizards. *Journal of Biogeography*, 49(12), 2193–2205. <https://doi.org/10.1111/jbi.14497>
- Eynan, M., & Dmi'el, R. (1993). Skin resistance to water loss in agamid lizards. *Oecologia*, 95, 290–294. <https://doi.org/10.1007/BF00323502>
- Fargallo, J. A., Navarro-López, J., Palma-Granados, P., & Nieto, R. M. (2020). Foraging strategy of a carnivorous-insectivorous raptor species based on prey size, capturability and nutritional components. *Scientific Reports*, 10(1), 7583. <https://doi.org/10.1038/s41598-020-64504-4>
- Farley, C. T. (1997). Maximum speed and mechanical power output in lizards. *Journal of Experimental Biology*, 200(16), 2189–2195. <https://doi.org/10.1242/jeb.200.16.2189>
- Farley, C. T., & Ko, T. C. (1997). Mechanics of locomotion in lizards. *Journal of Experimental Biology*, 200(16), 2177–2188. <https://doi.org/10.1242/jeb.200.16.2177>
- Ferreira, C. C., Santos, X., & Carretero, M. A. (2016). Does ecophysiology mediate reptile responses to fire regimes? Evidence from Iberian lizards. *PeerJ*, 4, e2107. <https://doi.org/10.7717/peerj.2107>
- Fuller, P. O., Higham, T. E., & Clark, A. J. (2011). Posture, speed, and habitat structure: Three-dimensional hindlimb kinematics of two species of padless geckos. *Zoology*, 114(2), 104–112. <https://doi.org/10.1016/j.zool.2010.11.003>
- Galán, P. (2003). *Anfibios y Reptiles del Parque Nacional de las Islas Atlánticas de Galicia*. Naturaleza y Parques Nacionales. Serie Técnica, Ministerio de Medio Ambiente, Madrid.
- García-Porta, J., Irisarri, I., Kirchner, M., Kirchhof, S., Brown, J. L., MacLeod, A., Turner, A. P., Ahmadzadeh, F., Albadalejo, G., Crnobrnja-Isailović, J., de la Riva, I., Fawzi, A., Galán, P., Göçmen, B., Harris, D. J., Jiménez-Robles, O., Joger, U., Jovanović-Glavavaš, O., Kariş, M., ... Wollemberg-Valero, K. C. (2019). Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nature Communications*, 10, 4077. <https://doi.org/10.1038/s41467-019-11943-x>
- Garland, T., Jr., & Losos, J. B. (1994). Ecological morphology of locomotor performance in squamate reptiles. In T. Garland, Jr. & J. B. Losos (Eds.), *Ecological morphology: Integrative organismal biology* (pp. 240–302). University of Chicago Press.
- Gomes, V., Carretero, M. A., & Kaliontzopoulou, A. (2016). The relevance of morphology for habitat use and locomotion in two species of wall lizards. *Acta Oecologica*, 70, 87–95. <https://doi.org/10.1016/j.actao.2015.12.005>
- Gomes, V., Carretero, M. A., & Kaliontzopoulou, A. (2018). Run for your life, but bite for your rights? How interactions between natural and sexual selection shape functional morphology across habitats. *The Science of Nature*, 105, 1–12. <https://doi.org/10.1007/s00114-017-1537-6>
- Gomes, V., Herrel, A., Carretero, M. A., & Kaliontzopoulou, A. (2020). New insights into bite performance: Morphological trade-offs underlying the duration and magnitude of bite force. *Physiological and Biochemical Zoology*, 93(3), 175–184. <https://doi.org/10.1086/708248>
- Goodman, B. A., Miles, D. B., & Schwarzkopf, L. (2008). Life on the rocks: Habitat use drives morphological and performance evolution in lizards. *Ecology*, 89(12), 3462–3471. <https://doi.org/10.1890/07-2093.1>
- Gravel, D., Guichard, F., & Hochberg, M. E. (2011). Species coexistence in a variable world. *Ecology Letters*, 14(8), 828–839. <https://doi.org/10.1111/j.1461-0248.2011.01643.x>
- Grinnell, J. (1917). The niche-relationships of the California thrasher. *Auk*, 34(4), 427–433. <https://doi.org/10.2307/4072271>
- Hastie, T., & Tibshirani. (2023). *mda: Mixture and flexible discriminant analysis*. R package version 0.5-4. <https://search.r-project.org/CRAN/refmans/mda/html/fda.html>
- Herrel, A., Damme, R. V., Vanhooydonck, B., & Vree, F. D. (2001). The implications of bite performance for diet in two species of lacertid lizards. *Canadian Journal of Zoology*, 79(4), 662–670. <https://doi.org/10.1139/z01-031>
- Herrel, A., Podos, J., Huber, S. K., & Hendry, A. P. (2005). Bite performance and morphology in a population of Darwin's finches: Implications for the evolution of beak shape. *Functional Ecology*, 19(1), 43–48. <https://doi.org/10.1111/j.0269-8463.2005.00923.x>
- Herrel, A., Van Damme, R., Vanhooydonck, B., Zaaf, A., & Aerts, P. (2000). Lizard locomotion: How morphology meets ecology. *Netherlands Journal of Zoology*, 50(2), 261–277. <https://doi.org/10.1163/15685420050865>
- Ho, H. C., Tylianakis, J. M., & Pawar, S. (2021). Behaviour moderates the impacts of food-web structure on species coexistence. *Ecology Letters*, 24(2), 298–309. <https://doi.org/10.1111/ele.13643>
- Holt, R. D. (2009). Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences of the United States of America*, 106(supplement_2), 19659–19665. <https://doi.org/10.1073/pnas.0905137106>
- Horn, M. H., & Riegle, K. C. (1981). Evaporative water loss and intertidal vertical distribution in relation to body size and morphology of stichaeoid fishes from California. *Journal of Experimental Marine Biology and Ecology*, 50(2–3), 273–288. [https://doi.org/10.1016/0022-0981\(81\)90054-X](https://doi.org/10.1016/0022-0981(81)90054-X)
- Hothorn, T., Müller, J., Schröder, B., Kneib, T., & Brandl, R. (2011). Decomposing environmental, spatial, and spatiotemporal components of species distributions. *Ecological Monographs*, 81(2), 329–347. <https://doi.org/10.1890/10-0602.1>
- Hutchinson, G. E. (1957). Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, 22, 415–427.
- Hutchinson, G. E. (1978). *An introduction to population ecology*. Yale University Press.
- Huyghe, K., Vanhooydonck, B., Scheers, H., Molina-Borja, M., & Van Damme, R. (2005). Morphology, performance and fighting capacity in male lizards, *Gallotia galloti*. *Functional Ecology*, 19, 800–807. <https://doi.org/10.1111/j.1365-2435.2005.01038.x>
- Irschick, D. J., & Losos, J. B. (1998). A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean

- Anolis lizards. *Evolution*, 52(1), 219–226. <https://doi.org/10.1111/j.1558-5646.1998.tb05155.x>
- Irschick, D. J., Meyers, J. J., Husak, J. F., & Le Galliard, J. F. (2008). How does selection operate on whole-organism functional performance capacities? A review and synthesis. *Evolutionary Ecology Research*, 10(2), 177–196. <https://doi.org/10.7275/R58G8HX6>
- Kalontzopoulou, A., Adams, D. C., van der Meijden, A., Perera, A., & Carretero, M. A. (2012). Relationships between head morphology, bite performance and ecology in two species of *Podarcis* wall lizards. *Evolutionary Ecology*, 26, 825–845. <https://doi.org/10.1007/s10682-011-9538-y>
- Kassambara, A. (2016). *Factoextra: Extract and visualize the results of multivariate data analyses*. R package version, 1.
- Kearney, M. (2006). Habitat, environment and niche: What are we modelling? *Oikos*, 115(1), 186–191. <https://doi.org/10.1111/j.2006.0030-1299.14908.x>
- Kearney, M., & Porter, W. (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters*, 12(4), 334–350. <https://doi.org/10.1111/j.1461-0248.2008.01277.x>
- Kearney, M. R., Munns, S. L., Moore, D., Malishev, M., & Bull, C. M. (2018). Field tests of a general ectotherm niche model show how water can limit lizard activity and distribution. *Ecological Monographs*, 88(4), 672–693. <https://doi.org/10.1002/ecm.1326>
- Kearney, M. R., & Porter, W. P. (2017). NicheMapR—An R package for biophysical modelling: The microclimate model. *Ecography*, 40(5), 664–674. <https://doi.org/10.1111/ecog.04680>
- Kohlsdorf, T., & Navas, C. A. (2006). Ecological constraints on the evolutionary association between field and preferred temperatures in Tropidurinae lizards. *Evolutionary Ecology*, 20, 549–564. <https://doi.org/10.1007/s10682-006-9116-x>
- Kraft, N. J., Valencia, R., & Ackerly, D. D. (2008). Functional traits and niche-based tree community assembly in an Amazonian forest. *Science*, 322(5901), 580–582. <https://doi.org/10.1126/science.1160662>
- Lappin, A. K., & Husak, J. F. (2005). Weapon performance, not size, determines mating success and potential reproductive output in the collared lizard (*Crotaphytus collaris*). *The American Naturalist*, 166(3), 426–436. <https://doi.org/10.1086/432564>
- Larson, D. W. (1984). Habitat overlap/niche segregation in two Umbilicaria lichens: A possible mechanism. *Oecologia*, 62, 118–125. <https://doi.org/10.1007/BF00377384>
- Laube, I., Kornthauer, H., Schwager, M., Trautmann, S., Rahbek, C., & Böhning-Gaese, K. (2013). Towards a more mechanistic understanding of traits and range sizes. *Global Ecology and Biogeography*, 22(2), 233–241.
- Le Galliard, J. F., Chabaud, C., de Andrade, D. O. V., Brischoux, F., Carretero, M. A., Dupoué, A., Gavira, R. S. B., Lourdais, O., Sannolo, M., & Van Dooren, T. J. (2021). A worldwide and annotated database of evaporative water loss rates in squamate reptiles. *Global Ecology and Biogeography*, 30(10), 1938–1950. <https://doi.org/10.1111/j.1466-8238.2012.00798.x>
- Le Lagadec, M. D., Chown, S. L., & Scholtz, C. H. (1998). Desiccation resistance and water balance in southern African keratin beetles (Coleoptera, Trogidae): The influence of body size and habitat. *Journal of Comparative Physiology B*, 168, 112–122. <https://doi.org/10.1007/s003600050127>
- Llorente, G. A., Montori, A., Santos, X., & Carretero, M. A. (1995). *Atlas de distribució dels Anfibis y Réptils de Catalunya y Andorra*. El Grau, Figueres: 192 pp.
- Loureiro, A., Ferrand, N., Carretero, M. A., & Paulo, O. (Eds.). (2008). *Atlas dos Anfíbios e Répteis de Portugal*. Instituto da Conservação da Natureza e da Biodiversidade.
- Lu, X., Gong, G., & Ma, X. (2011). Niche segregation between two alpine rosefinches: To coexist in extreme environments. *Evolutionary Biology*, 38, 79–87. <https://doi.org/10.1007/s11692-010-9102-7>
- Marco, A., & Pollo, C. (1993). Análisis biogeográfico de la distribución del lagarto verdinegro (*Lacerta schreiberi* Bedriaga 1878). *Ecología*, 7, 457–466.
- Martin, P. R., & Martin, T. E. (2001). Behavioral interactions between two coexisting wood warblers (Parulidae: Vermivora): Experimental and empirical tests. *Ecology*, 82, 207–218. <https://doi.org/10.2307/2680097>
- Martínez-Freiría, F., Lizana, M., do Amaral, J. P., & Brito, J. C. (2010). Spatial and temporal segregation allows coexistence in a hybrid zone among two Mediterranean vipers (*Vipera aspis* and *V. latastei*). *Amphibia-Reptilia*, 31(2), 195–212. <https://doi.org/10.1163/156853810791069001>
- Mateo, J. A. (2017). Lagarto ocelado—*Timon lepidus*. In A. Salvador & A. Marco (Eds.), *Enciclopedia Virtual de los Vertebrados Españoles* (pp. 1–52). Museo Nacional de Ciencias Naturales.
- Mateo, J. A., & Castanet, J. (1994). Reproductive strategies in three Spanish populations of the ocellated lizard, *Lacerta lepida* (Sauria, Lacertidae). *Acta Oecologica*, 1, 215–229.
- Mateo, J. A., & Cheylan, M. (1997). *Lacerta lepida* Daudin 1802. SEH & Muséum National d'Histoire Naturelle.
- Mautz, W. J. (1980). Factors influencing evaporative water loss in lizards. *Comparative Biochemistry and Physiology Part A: Physiology*, 67(3), 429–437. [https://doi.org/10.1016/S0300-9629\(80\)80019-3](https://doi.org/10.1016/S0300-9629(80)80019-3)
- McBryer, L. D., & White, T. D. (2002). Bite force, behavior, and electromyography in the teiid lizard, *Tupinambis teguixin*. *Copeia*, 2002(1), 111–119. [https://doi.org/10.1643/0045-8511\(2002\)002\[0111:BFBAE\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2002)002[0111:BFBAE]2.0.CO;2)
- McPeek, M. A. (2014). Limiting factors, competitive exclusion, and a more expansive view of species coexistence. *The American Naturalist*, 183(3), iii–iv. <https://doi.org/10.1086/675305>
- Mevik, B. H., Wehrens, R., & Liland, K. H. (2011). *pls: Partial least squares and principal component regression*. R package version, 2(3).
- Mi, C., Ma, L., Wang, Y., Wu, D., Du, W., & Sun, B. (2022). Temperate and tropical lizards are vulnerable to climate warming due to increased water loss and heat stress. *Proceedings of the Royal Society B: Biological Sciences*, 289(1980), 20221074. <https://doi.org/10.1098/rspb.2022.1074>
- Morales, M. M., & Giannini, N. P. (2010). Morphofunctional patterns in Neotropical felids: Species co-existence and historical assembly. *Biological Journal of the Linnean Society*, 100(3), 711–724. <https://doi.org/10.1111/j.1095-8312.2010.01461.x>
- Murren, C. J. (2012). The integrated phenotype. *Integrative and Comparative Biology*, 52(1), 64–76. <https://doi.org/10.1093/icb/ics043>
- Nagy, K. A. (2005). Field metabolic rate and body size. *Journal of Experimental Biology*, 208(9), 1621–1625. <https://doi.org/10.1242/jeb.01553>
- Navarro, J., Votier, S. C., Aguzzi, J., Chiesa, J. J., Forero, M. G., & Phillips, R. A. (2013). Ecological segregation in space, time and trophic niche of sympatric planktivorous petrels. *PLoS One*, 8(4), e62897. <https://doi.org/10.1371/journal.pone.0062897>
- Olalla-Tárraga, M. Á., Rodríguez, M. Á., & Hawkins, B. A. (2006). Broad-scale patterns of body size in squamate reptiles of Europe and North America. *Journal of Biogeography*, 33(5), 781–793.
- Pérez Mellado, V. (1982). Estructura en una taxocenosis de Lacertidae (Sauria, Reptilia) del sistema central. In *Mediterránea. Serie de Estudios Biológicos*, N. 6 (diciembre 1982) (pp. 39–64). Universidad de Alicante.
- Peterson, C. C., & Husak, J. F. (2006). Locomotor performance and sexual selection: Individual variation in sprint speed of collared lizards (*Crotaphytus collaris*). *Copeia*, 2006(2), 216–224. [https://doi.org/10.1643/0045-8511\(2006\)6\[216:LPASSI\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2006)6[216:LPASSI]2.0.CO;2)
- Phillips, B. L., Muñoz, M. M., Hatcher, A., Macdonald, S. L., Llewelyn, J., Lucy, V., & Moritz, C. (2016). Heat hardening in a tropical lizard: Geographic variation explained by the predictability and variance in environmental temperatures. *Functional Ecology*, 30(7), 1161–1168. <https://doi.org/10.1111/1365-2435.12609>

- Pianka, E. R., & Huey, R. B. (1978). Comparative ecology, resource utilization and niche segregation among gekkonid lizards in the southern Kalahari. *Copeia*, 1978, 691–701. <https://doi.org/10.2307/1443698>
- Pigot, A. L., Trisos, C. H., & Tobias, J. A. (2016). Functional traits reveal the expansion and packing of ecological niche space underlying an elevational diversity gradient in passerine birds. *Proceedings of the Royal Society B: Biological Sciences*, 283(1822), 20152013. <https://doi.org/10.1098/rspb.2015.2013>
- Prins, H. H., De Boer, W. F., Van Oeveren, H., Correia, A., Mafuca, J., & Olff, H. (2006). Co-existence and niche segregation of three small bovid species in southern Mozambique. *African Journal of Ecology*, 44(2), 186–198. [https://doi.org/10.1643/0045-8511\(2006\)6\[216:Lpassi\]2.0.Co;2](https://doi.org/10.1643/0045-8511(2006)6[216:Lpassi]2.0.Co;2)
- Quinn, T. P., & Foote, C. J. (1994). The effects of body size and sexual dimorphism on the reproductive behaviour of sockeye salmon, *Oncorhynchus nerka*. *Animal Behaviour*, 48(4), 751–761. <https://doi.org/10.1371/journal.pone.0216448>
- R Core Team. (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Redtfeldt, R. A., & Davis, S. D. (1996). Physiological and morphological evidence of niche segregation between two co-occurring species of *Adenostoma* in California chaparral. *Ecoscience*, 3(3), 290–296. <https://doi.org/10.1080/11956860.1996.11682345>
- Renet, J., Dokhelar, T., Thirion, F., Tatin, L., Pernollet, C. A., & Bourgault, L. (2022). Spatial pattern and shelter distribution of the ocellated lizard (*Timon lepidus*) in two distinct Mediterranean habitats. *Amphibia-Reptilia*, 43(3), 263–276.
- Salvador, A. (1988). Selección de microhabitat del lagarto verdinegro (*Lacerta schreiberi*) (Sauria: Lacertidae). *Amphibia-Reptilia*, 9(3), 265–275. <https://doi.org/10.1163/156853888X00350>
- Salvidio, S., Calvi, G., Lamagni, L., & Gardini, G. (2006). Primi dati sulla dieta della lucertola ocellata *Timon lepidus* (Daudin, 1802) in Italia. *Acta Herpetologica*, 1(1), 73–76. https://doi.org/10.13128/Acta_Herpetol-1253
- Sannolo, M., Barroso, F. M., & Carretero, M. A. (2018). Physiological differences in preferred temperatures and evaporative water loss rates in two sympatric lacertid species. *Zoology*, 126, 58–64.
- Sannolo, M., Ponti, R., & Carretero, M. A. (2019). Waitin' on a sunny day: Factors affecting lizard body temperature while hiding from predators. *Journal of Thermal Biology*, 84, 146–153. <https://doi.org/10.1016/j.jtherbio.2019.07.001>
- Schoener, T. W. (1974). Resource partitioning in ecological communities: Research on how similar species divide resources helps reveal the natural regulation of species diversity. *Science*, 185(4145), 27–39. <https://doi.org/10.1126/science.185.4145.27>
- Sears, M. W., & Angilletta, M. J., Jr. (2004). Body size clines in *Sceloporus* lizards: Proximate mechanisms and demographic constraints. *Integrative and Comparative Biology*, 44(6), 433–442. <https://doi.org/10.1093/icb/44.6.433>
- Sillero, N., & García-Muñoz, E. (2010). Two new types of noose for capturing herps. *Acta Herpetologica*, 5, 259–263. https://doi.org/10.13128/Acta_Herpetol-9033
- S'khifa, A., Carretero, M. A., Harris, D. J., & Slimani, T. (2022). Ecophysiological conservativeness and size-mediated plasticity in the High Mountain lizard *Atlantolacerta andreanskyi* confirm its vulnerability to climate change. *Salamandra*, 58(2), 139–150.
- S'khifa, A., Koziel, G., Vences, M., Carretero, M. A., & Slimani, T. (2020). Ecophysiology of a lacertid community in the high Moroccan mountains suggests conservation guidelines. *Journal of Thermal Biology*, 94, 102743. <https://doi.org/10.1016/j.jtherbio.2020.102743>
- Smart, H. J., & Gee, J. H. (1979). Coexistence and resource partitioning in two species of darters (Percidae), *Etheostoma nigrum* and *Percina maculata*. *Canadian Journal of Zoology*, 57(10), 2061–2071. <https://doi.org/10.1139/z79-272>
- Stevenson, R. D., Peterson, C. R., & Tsuji, J. S. (1985). The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiological Zoology*, 58(1), 46–57. <https://doi.org/10.1086/physzool.58.1.30161219>
- Streinzer, M., Huber, W., & Spaethe, J. (2016). Body size limits dim-light foraging activity in stingless bees (Apidae: Meliponini). *Journal of Comparative Physiology A*, 202, 643–655. <https://doi.org/10.1007/s00359-016-1118-8>
- Stubbs, W. J., & Bastow Wilson, J. (2004). Evidence for limiting similarity in a sand dune community. *Journal of Ecology*, 92(4), 557–567. <https://doi.org/10.1111/j.0022-0477.2004.00898.x>
- Tobalske, B. W., & Dial, K. P. (2000). Effects of body size on take-off flight performance in the Phasianidae (Aves). *Journal of Experimental Biology*, 203(21), 3319–3332. <https://doi.org/10.1242/jeb.203.21.3319>
- Tokeshi, M. (2009). *Species coexistence: Ecological and evolutionary perspectives*. John Wiley & Sons.
- Valladares, F., Bastias, C. C., Godoy, O., Granda, E., & Escudero, A. (2015). Species coexistence in a changing world. *Frontiers in Plant Science*, 6, 866. <https://doi.org/10.3389/fpls.2015.00866>
- Van der Meij, M. A. A., & Bout, R. G. (2006). Seed husking time and maximal bite force in finches. *Journal of Experimental Biology*, 209(17), 3329–3335. <https://doi.org/10.1242/jeb.02379>
- Vanhooydonck, B., & Van Damme, R. (2003). Relationships between locomotor performance, microhabitat use and antipredator behaviour in lacertid lizards. *Functional Ecology*, 17, 160–169.
- Vasil'ev, A. G. (2021). The concept of morphoniche in evolutionary ecology. *Russian Journal of Ecology*, 52(3), 173–187. <https://doi.org/10.1134/S1067413621030097>
- Vasilopoulou-Kampitsi, M. (2020). *Manoeuvrability and the anatomy of the inner ear in lacertid lizards. An ecological approach*. PhD thesis, University of Antwerp.
- Verwijnen, D., Van Damme, R., & Herrel, A. (2002). Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. *Functional Ecology*, 16(6), 842–850. <https://doi.org/10.1046/j.1365-2435.2002.00696.x>
- Webber, Q. M., & McGuire, L. P. (2022). Heterothermy, body size, and locomotion as ecological predictors of migration in mammals. *Mammal Review*, 52(1), 82–95. <https://doi.org/10.1111/mam.12263>
- Wells, M. S. (1988). Effects of body size and resource value on fighting behaviour in a jumping spider. *Animal Behaviour*, 36(2), 321–326. [https://doi.org/10.1016/S0003-3472\(88\)80001-0](https://doi.org/10.1016/S0003-3472(88)80001-0)
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., ... Yutani, H. (2019). Welcome to the tidyverse. *Journal of Open-Source Software*, 4(43), 1686. <https://doi.org/10.21105/joss.01686>
- Williams, E. E. (1983). Ecomorphs, faunas, Island size, and diverse end points in Island radiations of *Anolis*. In R. B. Huey, E. R. Pianka, & T. W. Schoener (Eds.), *Lizard ecology: Studies of a model organism* (pp. 326–370). Harvard University Press.
- Wojczyński, M. K., & Tiwari, H. K. (2008). Definition of phenotype. *Advances in Genetics*, 60, 75–105. [https://doi.org/10.1016/S0065-2660\(07\)00404-X](https://doi.org/10.1016/S0065-2660(07)00404-X)
- Žagar, A., Carretero, M. A., & de Groot, M. (2022). Time changes everything: A multispecies analysis of temporal patterns in evaporative water loss. *Oecologia*, 198(4), 905–915.
- Žagar, A., Carretero, M. A., Vrezec, A., Drašler, K., & Kaliontzopoulou, A. (2017). Towards a functional understanding of species coexistence: Ecomorphological variation in relation to whole-organism performance in two sympatric lizards. *Functional Ecology*, 31(9), 1780–1791. <https://doi.org/10.1111/1365-2435.12878>
- Zeng, X., & Lu, X. (2009). Interspecific dominance and asymmetric competition with respect to nesting habitats between two snow finch species in a high-altitude extreme environment. *Ecological Research*, 24, 607–616. <https://doi.org/10.1007/s11284-008-0530-0>

Zobel, M. (1992). Plant species coexistence: The role of historical, evolutionary and ecological factors. *Oikos*, 65, 314–320. <https://doi.org/10.2307/3545024>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1: Compressed archive of Supplementary Material (SM1-SM7).

How to cite this article: Reyes-Puig, C., Enriquez-Urzelai, U., Carretero, M. A., & Kaliontzopoulou, A. (2024). Is it all about size? Dismantling the integrated phenotype to understand species coexistence and niche segregation. *Functional Ecology*, 38, 2350–2368. <https://doi.org/10.1111/1365-2435.14646>