



# Is ecophysiology congruent with the present-day relictual distribution of a lizard group? Evidence from preferred temperatures and water loss rates

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We assessed whether ecophysiological requirements are consistent with the environmental traits within the current ranges in a relictual lizard group, *Algyroides*, composed of four species with restricted and disjunctive distributions. We considered temperature, precipitation and their seasonal profiles, and focused on the preferred body temperature ( $T_p$ ) and the evaporative water loss (EWL). The ranges of all four species differed in environmental traits. The two geographically more restricted species followed divergent patterns: *A. moreoticus* inhabits hot and climatically buffered areas, and *A. marchi* occupies cold seasonal environments.  $T_p$  and EWL also differed among species following a geographical grouping: *A. nigropunctatus* (Slovenia) and *A. fitzingeri* (Sardinia) selected for lower  $T_p$  and lost less water than the southern species *A. moreoticus* (Peloponnese) and *A. marchi* (Southern Spain).  $T_p$  and EWL were correlated at species level but not at individual level within species. Results suggest that the current distribution of *Algyroides* species partly reflects their ecophysiology, with water ecology taking precedence over thermal ecology as constraining factor. By unravelling the environmental factors limiting the distribution of species, ecophysiology may provide directions for conservation, predicting the degree of vulnerability to climate change.

*Key words:* *Algyroides*, biogeography, Lacertidae, thermal preference, water loss

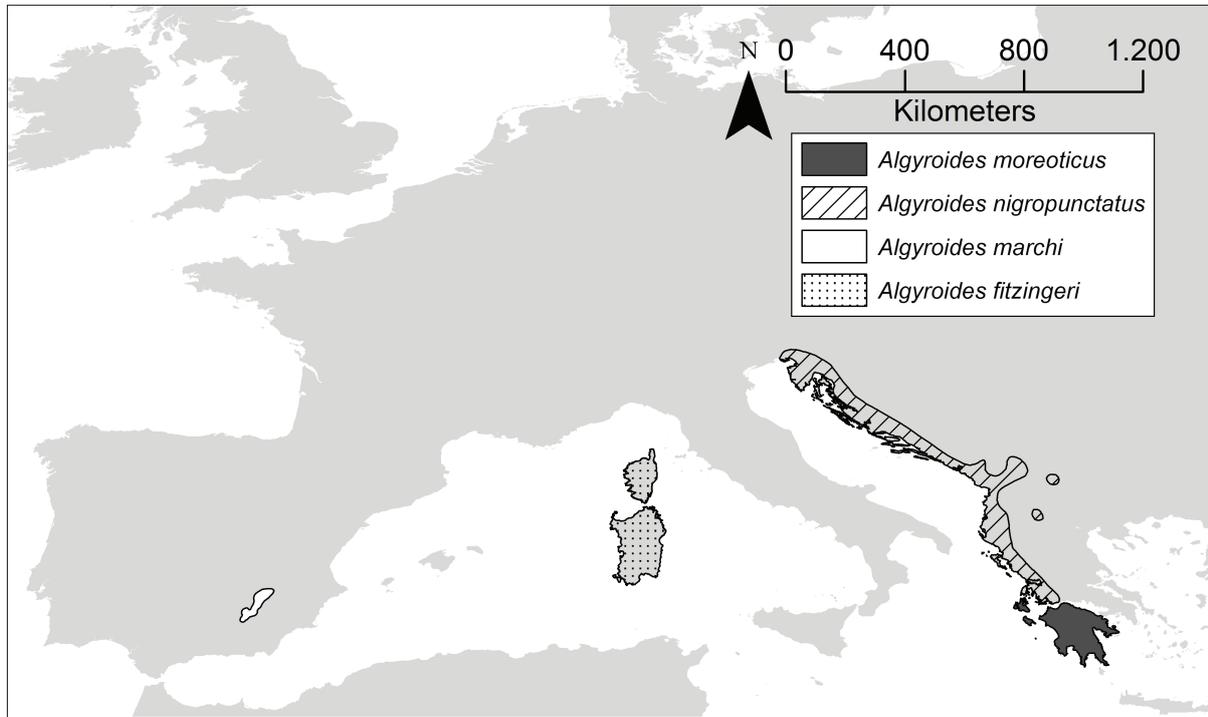
## INTRODUCTION

Animal distribution patterns attracted early researchers (i.e. Darwin, 1859). Biogeographic processes, historical or contemporary, are responsible for current ranges and are of crucial importance to interpret evolutionary processes (Templeton, 2002; Poulakakis et al., 2013). Studies integrating molecular and ecological data are widely used to reconstruct past distributions and explain contemporary ranges (Avice, 2000; Poulakakis et al., 2008). While phylogeography provides inference on the historical processes, comparative evidence on their current ecophysiological requirements is also needed for a mechanistic understanding of species distribution ranges. Even if such traits can be indirectly inferred through ecological models (Sillero, 2011; Warren, 2013), obtaining direct evidence on the organismal ecophysiology provides higher predictive power (Kearney et al., 2010; Rato & Carretero, 2015). Unfortunately, comparative ecophysiology remained largely neglected in the past probably due to logistical constraints.

Organisms perform within a specific physiological window (Schmidt-Nielsen, 1997), outside of which they become physically impaired and even die (Gordon et al., 1982). Ecological conservativeness together with changing environments may result in range shifts and even speciation in the long run (Wiens, 2004), but may also lead to extinction. Thus, ecophysiology of species may play an important role in determining their distribution patterns (Pearman et al., 2007) while the opposite is also true – distributional patterns may select for certain physiological characteristics. This is especially true for ectotherms as reptiles due to low levels of homeostasis (Foufopoulos & Ives, 1999).

Lacertid lizards, being short-lived, highly sedentary and poor over-water dispersers, provide excellent models to disentangle biogeographical scenarios (Poulakakis et al., 2003; Hurston et al., 2009). A plethora of studies have already shed light on the phylogeography of Mediterranean lacertids (e.g., Poulakakis et al., 2005; Carranza et al., 2006; Salvi et al., 2010; Kaliontzopoulou et al., 2011). On the other hand, a growing body of

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**Fig. 1.** Distribution ranges of the four *Algyroides* species.

literature associates climate change and extinction risk in reptiles (e.g., Huey et al., 2010; Sinervo et al., 2010). However, attention for this is mainly focused on thermal preferences while neglecting hydric requirements. Mechanistic models based on biophysical traits represent a step forward for providing more realistic inferences of lizard distributions but only some explicitly incorporate hydric parameters and always in a subsidiary role (Fei et al., 2012a, b; Kearney et al., 2013).

Preferred body temperature ( $T_p$ , body temperature that animals achieve in the absence of thermoregulatory constraints, Huey & Bennett, 1987) provides reliable inference of the thermal requirements across a lizard's distribution range while having several practical advantages. In lacertids,  $T_p$  is ecologically relevant since it correlates directly with several physiological optima (Huey & Bennett, 1987; Bauwens et al., 1995), has a relatively narrow variation in lacertid lizards (Huey, 1982), is conservative in the phylogeny (Bauwens et al., 1995) and remains similar across conspecific populations under different climate regimes (Gvoždik & Castilla, 2001; Díaz et al., 2006; Yang et al., 2008). Moreover,  $T_p$  can be recorded in the lab under standardised conditions for a reasonable number of individuals (Osojnik et al., 2013). Certainly, at the individual level  $T_p$  may change as a function of time of the day, season, feeding activity, ontogeny or reproductive status (Castilla et al., 1999). Nevertheless, all these sources of bias can be successfully removed by restricting interspecific comparisons to the same class and time period (Carretero et al., 2005).

Much less is known on reptilian hydric ecophysiology, but there is evidence that evaporative water loss (EWL) differs between species from xeric and humid habitats (Mautz, 1982b; Eynan & Dmi'el, 1993) indicating either a current or past adaptation. In reptiles, EWL mainly occurs through the skin but also through the respiratory passages

and the eyes (Mautz, 1982a). Hence EWL is potentially informative on the hydric constraints of fundamental niche, particularly under restricted water availability (Bowker et al., 1993).

Lacertid distributions cover the entire Mediterranean Basin where species frequently overlap (Arnold et al., 2007) but those with similar size and spatial requirements tend to be parapatric (Arnold, 1987). This suggests that their range distributions may be more driven by mean physiological values and species interactions rather than by critical values. This is why we favoured using means for assessing thermal and hydric ecophysiology.

In this study we explored the concordance between the thermal preferences and the water loss rates together with the distribution and habitat use in a small lizard group. The genus *Algyroides* is composed of four species with disjunct distributions across Europe (Arnold & Ovenden, 2002, Fig. 1). Although heliothermic as other Mediterranean lacertids, *Algyroides* species differ from them by inhabiting relatively shaded and humid areas which are scarce in the region (Arnold, 1987). This suggests thermals and/or hydric constraints (Arnold & Ovenden, 2002; Bressi, 2004). Our aim was to evaluate the importance of temperature and humidity on the distribution of this genus by assessing trends of thermal and hydric ecophysiology estimated from  $T_p$  and EWL. We hypothesised that the prevailing environmental conditions (a proxy for distribution) and these two traits should be congruent. First, we predicted that species living in colder environments would select for lower  $T_p$  according to their biological functions (Meiri et al., 2013). Second, we expected that species from humid environments would lose more water (higher EWLs) than those from more arid regions. Finally, we tried to clarify whether there are any trade-offs between the two ecophysiological traits,  $T_p$  and EWL, as earlier suggested (Bowker, 1993).

## MATERIAL AND METHODS

### Study species

The genus comprises four species that vary considerably in range extent (Fig. 1). The Dalmatian *Algyroides* (*A. nigropunctatus*) is widely distributed across the Adriatic coastline from north-eastern Italy to Greece (Sillero et al., 2014). The Greek *Algyroides* (*A. moreoticus*) is endemic to the Peloponnese and some Ionian islands (Valakos et al., 2008). The Pygmy *Algyroides* (*A. fitzingeri*) is endemic to Corsica and Sardinia where it is cryptic but widespread (Sillero et al., 2014). Finally, the Spanish *Algyroides* (*A. marchi*) is restricted to a narrow mountain range in south-eastern Spain (Carretero et al., 2010). Early molecular (mtDNA) and morphological evidence suggest that the genus originated in Eastern Europe during the Miocene and spread to the west where those forms most recently diverged are found (Harris et al., 1999). The monophyly of the genus has recently been revalidated by a combined analysis of mitochondrial and nuclear markers (Mendes et al., 2016).

In contrast to many other Mediterranean lacertids, all four *Algyroides* species share many original ecological and morphological features. Namely, they are small-bodied (snout-to-vent length 3–7 cm) and slender, with relatively short tails, small, narrow heads and keeled scales (Arnold & Ovenden, 2002). They tend to be associated with vegetation and shady environments, displaying mainly ground-dwelling habits (except in the rock climber *A. marchi*, Arnold, 1987).

### Environmental characterisation of geographic ranges

Since accurate records evenly distributed across the ranges were not available for all species, spatial distribution had to be characterised at a geographical scale based on the species' ranges. Even if approximate, the downscaling of spatial inferences has already been proved to be ecologically meaningful at least for *A. marchi* (Rubio & Carrascal, 1994). Thus, shapes for the range perimeter of each species were downloaded from the IUCN red list <<http://maps.iucnredlist.org>>. To quantitatively examine the thermal and hydric environment inside each range several representative bioclimatic variables were downloaded from the *Worldclim* database <<http://www.worldclim.org/bioclim>>; Hijmans et al., 2005), namely, annual

mean temperature, temperature seasonality, annual precipitation and precipitation seasonality (Table 1).

### Field sampling

In May (peak of the breeding season for all species), we captured adult male individuals from each species by hand or noose (García-Muñoz & Sillero, 2010) in representative localities of each species in Slovenia (*A. nigropunctatus*, Lake Vanganel, N 45°30', E 13°46'), Greece (*A. moreoticus*, Parnonas Mt, N 37° 17', E 22° 45'), Sardinia (*A. fitzingeri* 3 km east of Sassari (N 40°43', E 8°37'18) and Spain (*A. marchi*, Rambla los Vaquerizos, N 38°3', E -2°29'). Lizards were kept in individual plastic terraria (dimensions 20×40×25 cm) without exceeding five days in captivity with water and food (*Tenebrio molitor* larvae) provided *ad libitum* daily. For each individual, we recorded the SVL (to the nearest 0.01 mm with digital callipers) and the body weight ( $W_0$ , to the nearest 0.0001 g with a digital scale) immediately before the experimental procedure. All lizards were released after the experiments in the collecting sites.

### Preferred temperatures

To determine the  $T_p$  of each species, individual experiments with the use of a linear thermal gradient were conducted with a total of 33 lizards (nine *A. nigropunctatus*, twelve *A. moreoticus*, seven *A. fitzingeri* and five *A. marchi*). The different, and rather small, sample sizes were limited by the low population densities of this scarce, evasive genus (Bressi, 2004; Carretero et al., 2010). We only used regularly fed adult males to exclude eventual effects of vitellogenesis/pregnancy, low body condition and ontogeny on thermal preferences (Brown & Griffin 2003; Carretero et al., 2005). A natural regime of light and temperature was kept by sunlight coming through windows in the rooms and by infra-red light bulbs. One day prior to experiments lizards were not fed.

Each individual was placed in a terrarium (100×40×30 cm) with a substrate of a thin layer of soil without any shelter, food or water source. A thermal gradient ranging from 20 to 45°C was achieved by placing a 150W infrared reflector bulb at one end of the terrarium secured 25 cm above the substrate. The terraria were set in a room with natural light photoperiod and stable temperature around 15–20°C. Each lizard was introduced in the thermal gradient at 0800 hours (infra-red bulb was switched on at

**Table 1.** Environmental characterisation (Mean±SE) of the ranges of four species of *Algyroides*. *n*: number of environmental records downloaded from the *Worldclim* database (see Material and methods). Temperature seasonality: standard deviation (SD) of the weekly mean temperatures expressed as a percentage of the mean of those temperatures. Precipitation seasonality: coefficient of variation (CV) as the standard deviation of the weekly precipitation estimates expressed as a percentage of the annual mean.

Species	total area (km <sup>2</sup> )	<i>n</i>	annual mean temperature (°C)	temperature seasonality (SD*100)	annual precipitation (mm)	precipitation seasonality (CV)
<i>A. nigropunctatus</i>	68861	6622	12.52±0.04	6371.18±4.80	1153.62±2.40	36.02±0.19
<i>A. moreoticus</i>	23143	31794	14.75±0.01	5828.07±1.67	786.03±0.65	68.51±0.04
<i>A. fitzingeri</i>	35020	49396	14.31±0.01	5342.11±1.19	659.43±0.58	51.17±0.03
<i>A. marchi</i>	3643	7136	11.67±0.02	6587.93±1.12	536.17±1.02	36.10±0.04

0700) and left to freely thermoregulate for 11 hours that correspond to its daily activity period (Arnold, 1987). The lizard could freely select the most adequate temperature along time since there were no other thermoregulatory constraints (Veríssimo & Carretero, 2009). We measured cloacal temperatures every hour for ten consecutive hours (0900–1900) using a type-k thermocouple HIBOK® 18 (precision 0.1°C) inside a 1.5 mm diameter probe inserted in the cloaca. Lizard manipulation did not exceed 10 seconds to minimise stress and ensure more accurate temperature readings (Veríssimo & Carretero 2009). Alternative methods as cloacal probes (Clusella-Trullas et al., 2007) or infrared pistols (Carretero, 2012) would not be appropriate for these small, slender lizards. On the other hand, even though some authors suggested that disentangling between thermal preference and thermal dependence of movement in small ectotherms may be difficult (Dillon et al., 2012), here no obvious differences in activity between species were observed.

### Water-loss rates

The rates of EWL were determined for a total of 32 lizards (nine *A. nigropunctatus*, twelve *A. moreoticus*, six *A. fitzingeri* and five *A. marchi*). These experiments were always performed after preferred body temperature experiments and with two days in-between when lizards were kept in the housing terraria. Lizards were fed on the first day and fasted on the next day before the experiment. Each individual was placed inside a closed chamber (15x10x10 cm) with small ventilation holes and a false bottom containing five grams of silica gel. Individual chambers were placed inside a bigger and opaque sealed chamber (40x30x20 cm) under constant humidity conditions (~25%) ensured by 100 g silica gel placed under the chamber lid (Osojnik et al. 2013; Carneiro et al. 2015). The experiments were conducted under room temperature (~24°C) and low humidity conditions (20–30%) to standardize both factors. According to the known field temperatures of most lacertids (Castilla et al., 1999), 24°C falls around the low threshold of activity, which allowed lizards to be moderately active without being stressed inside a dark closed chamber, thus, providing biologically relevant measurements of

EWL. Room temperature and humidity were monitored every hour with a Fluke®-971 hygrothermometer to the nearest 0.1°C and 0.1%, respectively.

After one hour, each individual chamber (lizard with the chamber but without the false bottom) was weighted using a digital balance (precision 0.0001 g). Measurements were taken every hour for 12 consecutive hours (from 0700 to 1800 hours, solar time), which encompasses their daily activity period (Arnold, 1987). Each weighing operation took no more than 10 seconds, to minimise the disturbance of animals (Osojnik et al., 2013). Even though lizards were fasted one day prior to experiments, some of them defecated during the experiments. All measurements of those individuals were excluded prior to analysis.

### Statistical analyses

To test whether thermal and hydric conditions differed between distribution ranges of the four species we performed MANOVA comparisons on the multivariate dataset of temperature and precipitation separately, as well as ANOVA comparisons on single variables. All ecophysiological data followed a normal (Shapiro-Wilk test,  $p>0.05$ ), homoscedastic (multivariate Box M and univariate Levene's tests,  $p>0.05$ ) and non-spherical (Mauchly's tests,  $p>0.05$  in all cases) distribution.

SVL differences between species were determined with ANOVA followed by post-hoc tests using SVL as a dependent variable and species as an independent variable. Robustness (mass relative to length) of species was assessed with ANOVA using  $W_0$  as dependent variable and SVL as covariate.

In order to address putative differences in thermal preferences among species across time we performed repeated measures ANOVA (RM-ANOVA). SVL and initial weight ( $W_0$ ) were used as covariates in subsequent ANCOVAs in order to test for possible size, shape and body condition effects. Differences in the patterns of EWL rates among species were assessed using RM-ANOVA for instantaneous EWL relative to  $W_0$  ( $EWL_t$ ) and using \ species as the independent factor.  $EWL_t$  was calculated using the equation:  $EWL_t = [(W_n - W_{n+1}) / W_0]$  where  $W$  is the weight. To determine putative differences between

**Table 2.** Sample size ( $n$ ), mean snout-to-vent length (SVL), preferred body temperatures ( $T_p$ ), initial weight ( $W_0$ ) and total water loss ( $EWL_t$ ) of four *Algyroides* species.

Species	$n$	SVL (mm)	$T_p$ (°C)	$n$	$W_0$ (g)	$EWL_t$ (%)
		Mean±SE Min–Max	Mean±SE Min–Max		Mean±SE Min–Max	Mean±SE Min–Max
<i>A. nigropunctatus</i>	9	62.1±1.5	29.5±0.3	9	5.62±0.36	0.09±0.01
		54.4–68.7	28.5–31.0		3.84–6.56	0.05–0.14
<i>A. moreoticus</i>	12	47.9±0.4	31.7±0.2	12	3.18±0.23	0.14±0.01
		45.8–50.3	30.8–32.9		2.34–4.65	0.10–0.24
<i>A. fitzingeri</i>	7	37.2±0.9	28.8±0.2	6	1.16±0.11	0.06±0.01
		34.4–39.9	28.3–29.3		0.77–1.44	0.03–0.08
<i>A. marchi</i>	5	42.9±1.0	31.6±0.36	5	1.59±0.10	0.12±0.01
		41.7–44.1	30.9–32.3		1.32–1.87	0.08–0.15

species concerning their patterns of EWL in time, ANOVAs for each time interval were performed using accumulated EWL ( $EWL_a$ ) as the dependent variable and species as the independent variable.  $EWL_a$  was calculated using the equation:  $EWL_a = [(W_0 - W_n) / W_0]$ . The interaction between the mean  $T_p$  (calculated from 10 time interval measurements) and the total amount of water lost after the 12-hour experiment ( $EWL_t = [(W_0 - W_{11}) / W_0]$ ) was assessed with correlations between these factors at the species level (average of  $T_p$  and  $EWL_t$  by species) and at the individual level.

Statistica v. 12 (Statsoft Inc., 2013) was used to perform all analyses. Significance was evaluated at  $\alpha$ -value of 0.05.

## RESULTS

### Range characterisation

In terms of range extension, *A. nigropunctatus* occupied the largest range, followed by *A. fitzingeri*. *A. moreoticus* and especially *A. marchi* were restricted to very small areas (Table 1).

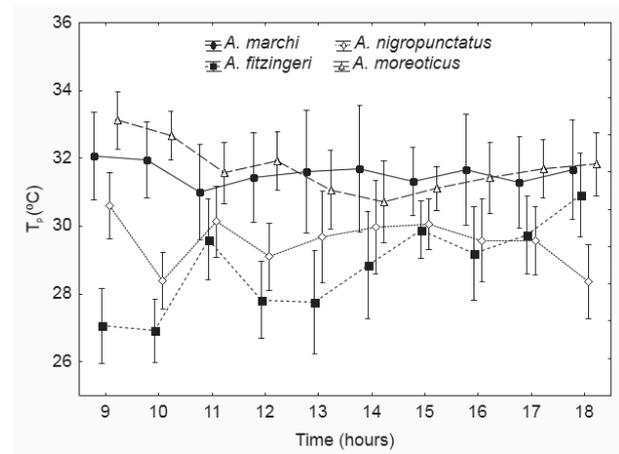
All four species strongly differed in their environmental niches at a multivariate level (MANOVA: Wilks Lambda  $_{12,246074} = 543405.14, p < 10^{-8}$ ). The same generally held true at a univariate level. The range of *A. moreoticus* achieved the highest annual mean temperature followed by *A. fitzingeri* and, at a longer distance, by *A. nigropunctatus* and *A. marchi* (ANOVA:  $F_{3,93056} = 4280.02, p < 10^{-8}$ ; Duncan tests  $p < 10^{-8}$ ). The widest temperature seasonality was found in *A. marchi* followed consecutively by *A. nigropunctatus*, *A. moreoticus* and *A. fitzingeri* (ANOVA:  $F_{3,93254} = 58154.37, p < 10^{-8}$ ; Duncan tests  $p < 10^{-8}$ ). In regard to the annual precipitation, species were sorted in decreasing order as *A. nigropunctatus*, *A. moreoticus*, *A. fitzingeri* and *A. marchi* (ANOVA:  $F_{3,93011} = 35335.22, p < 10^{-8}$ ; Duncan tests  $p < 10^{-8}$ ). Finally, the precipitation seasonality decreased from *A. moreoticus* to *A. fitzingeri* and to *A. nigropunctatus*-*A. marchi* (ANOVA:  $F_{3,94944} = 67473.46, p < 10^{-8}$ ; Duncan tests  $p < 10^{-8}$ ). The latter two species did not differ between them (Duncan test  $p = 0.96$ ).

### Basic morphometric characterisation

The four species (Table 2) differed in SVL ( $F_{3,29} = 124.49; p < 10^{-2}$ ) and robustness (weight accounting for SVL;  $F_{3,27} = 3.59; p = 0.03$ ). All species were different in length, sorted in order of increasing size as *A. fitzingeri*, *A. marchi*, *A. moreoticus* and *A. nigropunctatus* (Duncan tests,  $p < 0.001$ ). *Algyroides nigropunctatus* was the most robust followed by *A. moreoticus* and finally the most gracile *A. marchi* and *A. fitzingeri* (Duncan tests,  $p < 0.001$ ) which did not differ (Duncan test,  $p = 0.06$ ).

### Preferred temperatures

The thermal preferences of the four *Algyroides* species (Table 2) revealed interspecific differences in mean  $T_p$ . However, its temporal variation (Fig. 1) did not follow a common pattern (RM-ANOVA species  $F_{3,261} = 39.01; p < 10^{-6}$ ; time  $F_{9,261} = 1.03; p = 0.42$ ; species\*time  $F_{27,261} = 3.12; p = 10^{-6}$ ) except between *A. moreoticus* and *A. marchi* (Duncan test,  $p = 0.68$ ). Such results persisted after accounting for SVL (species  $F_{3,252} = 46.92; p < 10^{-6}$ ; time  $F_{9,252} = 1.68; p = 0.09$ ;



**Fig. 2.** Daily variations of the preferred body temperatures ( $T_p$ ) of four *Algyroides* species. Displayed are median values and 0.95 confidence intervals.

time\*species,  $F_{27,252} = 2.58, p = 0.00007$ ), and for both SVL and  $W_0$  (species  $F_{3,234} = 45.04; p < 10^{-6}$ ; time  $F_{9,234} = 0.75; p = 0.66$ ; species\*time  $F_{27,234} = 2.58, p = 0.00007$ ).

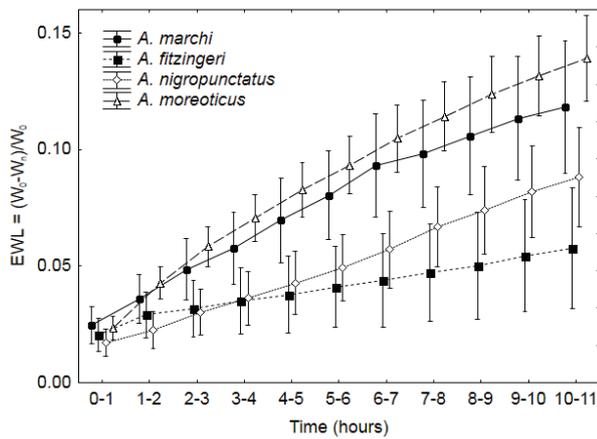
### Water loss rates

The  $EWL_t$  displayed complex patterns of variation between species (Table 2) and time profiles (Fig. 2).  $EWL_t$  varied not only between species and along time but also displayed different temporal patterns (RM-ANOVA species  $F_{3,280} = 10.58; p = 0.00008$ ; time  $F_{10,280} = 239.07; p < 10^{-7}$ ; species\*time  $F_{30,280} = 12.89; p < 10^{-7}$ ). Essentially, two groups (Duncan tests  $p < 0.02$ ) with no internal differences could be observed: *A. moreoticus* and *A. marchi* displayed high  $EWL_t$  rates, while *A. nigropunctatus* and *A. fitzingeri* attained low  $EWL_t$  rates (Table 2). Again, results remained similar after correcting for SVL (species  $F_{3,270} = 18.72; p = 10^{-7}$ ; time  $F_{10,270} = 6.41; p < 10^{-7}$ ; species\*time  $F_{30,270} = 20.71; p < 10^{-7}$ ), and for both SVL and  $W_0$  (species  $F_{3,260} = 19.92; p < 10^{-7}$ ; time  $F_{10,260} = 0.45; p = 0.91$ ; species\*time  $F_{30,260} = 25.21; p < 10^{-7}$ ), although time variation in the second case was weight-dependent (weight\*time  $F_{10,260} = 8.67; p < 10^{-7}$ ).

The analysis of the temporal variation of  $EWL_a$  of the four species (Fig. 2) and the statistical evaluation of  $EWL_a$  in each time interval (Appendix, Table 1) revealed the origin of such differences. Namely, the divergence between groups of high and low  $EWL_a$  gradually increased in time. Moreover, the patterns of *A. marchi* and *A. moreoticus* were similar while *A. nigropunctatus* tended to deviate towards higher values than *A. fitzingeri*, but only in the second half of the experiment (Fig. 2).

### Preferred temperatures vs. water loss rates

The relation between mean  $T_p$  and  $EWL_t$  at the species level was significant ( $n = 4, EWL_t = 0.023 T_p + 0.597; r^2 = 0.94, df = 3, p < 10^{-5}$ ). However, such a correlation was not supported at the individual level in any of the four species (*A. nigropunctatus*  $n = 9, A. moreoticus$   $n = 12, A. fitzingeri$   $n = 7, A. marchi$   $n = 5; p > 0.14$  in all cases). Neither SVL nor  $W_0$  had an influence on these results (multiple regression *A. nigropunctatus*  $F_{3,5} = 0.69, p = 0.60, A. moreoticus$   $F_{3,8} = 1.25, p = 0.35, A. fitzingeri$   $F_{3,2} = 1.98, p = 0.35, A. marchi$   $F_{3,1} = 2.12, p = 0.46$ ).



**Fig. 3.** Patterns of accumulated water loss ( $EWL_a$ ) along a 12-hour experiment for four *Algyroides* species. Displayed are median values and 0.95 confidence intervals.

## DISCUSSION

The genus *Algyroides* displays a relictual distribution pattern mainly associated with vegetated habitats (Arnold, 1987), a fact that contrasts with those of other lacertid genera ranging across the Mediterranean Basin (Arnold et al., 2007). The phylogenetic analyses (Harris et al., 1999; Mendes et al., 2016) support a gradual range retraction of the genus not only in coincidence with the expansion of *Podarcis* wall lizards, but also with the aridification and the establishment of the Mediterranean climate during the Pliocene (Cavazza & Wezel, 2003). Phylogeographic studies also indicate that during the Pleistocene *A. marchi* suffered a severe range retraction (Carretero et al., 2010) and *A. nigropunctatus* distribution was fragmented (Podnar & Mayer, 2006; Salvi et al. 2011) whereas the range of *A. fitzingeri* increased (Salvi et al., 2011). Unfortunately no equivalent data are available for *A. moreoticus*. All this evidence suggested that conservative physiological traits could be constraining the current biogeography of *Algyroides* and that differences between the species of this genus might be expected. Our analyses of the environmental range and ecophysiological traits support this niche conservatism, suggesting that water ecology plays a more critical role than thermal ecology while the linkage between thermal and hydric traits in this lizard group is unclear.

Even if approximate, our environmental estimations clearly demonstrated that geographic distributions of the four species differ in extent and climate. Interestingly, the two species occupying restricted ranges differed in the environmental traits assessed: *A. moreoticus* occurs in areas with high but less seasonal temperatures and precipitations whereas *A. marchi* is found in areas with relatively low and highly seasonal temperatures and precipitations. Even if derived from an environmental correlation, these findings suggest that similarities in range pattern between these Mediterranean lizards do not necessarily involve the same ecogeographical processes.

On the other hand, ecophysiological traits group the study species in two pairs: *A. nigropunctatus* and *A. fitzingeri* selected for lower  $T_p$  and lower EWL, while *A.*

*moreoticus* and *A. marchi* achieved higher  $T_p$  and EWL. Certainly, preferred temperatures may change with season, sex, age, reproductive status and body size within a single species (Carretero et al., 2005; Veríssimo & Carretero 2009; Sagonas et al., 2013a) but these factors were excluded in our experimental design. It is noteworthy that differences persisted even after accounting for the interspecific differences in size. Therefore, we have to conclude that intrinsic differences in ecophysiology between the four *Algyroides* species must exist.

Thermal preferences, as measured under laboratory conditions, have been traditionally interpreted as an inherent character (Van Damme et al., 1986). They represent the ideal body temperature, a “thermal utopia” (Sagonas et al., 2013b), under which many of the physiological processes of an ectotherm would reach their optimal performance (i.e. locomotion, Huey, 1982). As such, it has been proposed that thermal preferences may explain, at least partly, biogeographical patterns (Huey & Bennett, 1987). While our results do not contradict this assumption, they do not fully support it. In fact, strong signals across phylogenies (Bauwens et al., 1995) and thermal rigidity within species (Van Damme et al., 1986; Carretero et al., 2005; Díaz et al., 2006) have already been reported in lacertids. Nevertheless, convergence in thermal traits must be common in this family, since phylogenetically unrelated species and genera select for similar  $T_p$  (Bauwens et al., 1995). This seems to be the case in *Algyroides* since the most similar species in ecophysiology are not the closest in phylogeny (Harris et al., 1999; Mendes et al., 2016).

The pair of southern and the most restricted species had higher EWL rates compared to the pair of widely distributed species. Strikingly, under the same experimental conditions, *A. moreoticus* lost over twice the amount of water than *A. fitzingeri* during the same period. Two recent studies applying the same methods place the EWL rates of *Algyroides* among the highest ever recorded in the family Lacertidae (0.05–0.15 g in *Algyroides* vs. 0.03–0.05 g in *Podarcis* and *Iberolacerta*, Osojnik et al., 2013; Carneiro et al., 2015). EWL rates reflect the ability of an animal to retain the water and therefore it is considered an important indicator for the survival of lizards (Bowker, 1993). This is particularly relevant in Mediterranean environments where this resource is spatially, daily and seasonally restricted (Kaliontzopoulou et al., 2008). While the distribution ranges of *A. moreoticus* and *A. marchi* are biogeographically isolated within the Mediterranean region, they both tend to be more humid and less seasonally variable in precipitation than the surrounding areas. This, allied to the occupation (not studied here) of the more humid microhabitats (Mayer & Beyerlein, 1999; Valakos et al., 2008; Carretero et al., 2010), seems to have allowed *A. moreoticus* and *A. marchi* to survive during the most arid paleoclimatic periods (Carretero et al., 2010) but at the cost of becoming geographically localised and ecologically restricted. Considering the extremely low water availability in southern Iberia and the Peloponnese, the highest EWL rates observed for these two species do not reflect current adaptation to dry environment but likely result from historical constraints.

A positive relation between  $T_p$ s and EWLs exists among the four *Algyroides* species (if phylogeny is ignored). Since water evaporation is physically related with temperature, lizards with similar characteristics inhabiting warmer environments should lose more water. Because of that, a trade-off between both traits has been suggested at intraspecific level for thermoregulatory lizards (Mautz, 1982a; Bowker, 1993). However, the application of this rule to the present results at the interspecific level is problematic. Water should also be a priority for ectothermic species living in warm and arid environments (Polymeni et al., 2010) and species under high hydric stress should either evolve adaptations to prevent high water loss rates or conserve traits evolved in the past under these conditions. However, the statistical correction of the thermal and hydric variables for SVL and  $W_0$  in *Algyroides* rendered the same results as those uncorrected, which suggests conservatism in ecophysiology. A formal analysis under the comparative method framework cannot be performed with only a four-species group and should wait for a larger species dataset including other lacertid genera to confirm or reject this hypothesis.

From the conservation point of view, *A. moreoticus* and *A. marchi* inhabit regions where water availability is restricted but still they select for high temperatures. This puts them at an ecophysiological risk while their spatial distribution may be strongly restricted (Table 2). Thus, it is not surprising that both species attain high rankings on IUCN red lists (*A. moreoticus* Near Threatened, Böhme & Lymberakis, 2009; and *A. marchi* Endangered, Pérez-Mellado et al., 2009, Carretero et al., 2010). Inversely, *A. nigropunctatus* and *A. fitzingeri* are catalogued by IUCN as Least Concern (Böhme et al., 2009; Corti et al., 2009). Apparently the lower ecophysiological restriction, particularly in the water ecology, of *A. nigropunctatus* and *A. fitzingeri* makes them less constrained and is also reflected in a wider distribution.

Lately, ecophysiology is gaining ground as a useful tool to predict extinction risks (Wikelski and Cooke, 2008). On the basis of correlative ecological models, *A. marchi* has already been predicted to become extinct within this century if climate change scenarios are confirmed (Carvalho et al., 2010). In this context, ecophysiological data could provide with more accurate species-specific information on biogeography, which could be projected to both the past and the forecasted future (Huey et al., 2012). Combining evidence from phylogeography, climate shifts and ecophysiology could hence enable a better understanding on how species attain their current distribution patterns and how they may be modified by non-human and human impacts.

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## APPENDIX

**Table 1.** ANOVA of accumulated water loss (EWL<sub>a</sub>) assessed for 12 consecutive hours between species and sexes using the covariates SVL and W<sub>0</sub>.  $EWL_a = (W_0 - W_n) / W_0$ .

		$(W_0 - W_1) / W_0$		$(W_0 - W_2) / W_0$		$(W_0 - W_3) / W_0$		$(W_0 - W_4) / W_0$		$(W_0 - W_5) / W_0$		$(W_0 - W_6) / W_0$	
	df	F	p	F	p	F	p	F	p	F	p	F	p
Species	3, 28	1.23	0.32	5.56	0.004	8.41	0.0004	9.86	<10 <sup>-4</sup>	10.51	<10 <sup>-4</sup>	12.21	<10 <sup>-5</sup>
		$(W_0 - W_7) / W_0$		$(W_0 - W_8) / W_0$		$(W_0 - W_9) / W_0$		$(W_0 - W_{10}) / W_0$		$(W_0 - W_{11}) / W_0$			
	df	F	p	F	p	F	p	F	p	F	p		
Species	3, 28	11.89	<10 <sup>-5</sup>	12.021	<10 <sup>-5</sup>	11.59	0.00004	11.36	<10 <sup>-4</sup>	10.73	<10 <sup>-4</sup>		
		$(W_0 - W_1) / W_0$		$(W_0 - W_2) / W_0$		$(W_0 - W_3) / W_0$		$(W_0 - W_4) / W_0$		$(W_0 - W_5) / W_0$		$(W_0 - W_6) / W_0$	
Covariate	df	F	p	F	p	F	p	F	p	F	p	F	p
SVL	1, 27	4.07	0.05	7.11	0.01	8.73	0.01	8.05	0.01	9.58	0.01	9.00	0.01
Species	3, 27	2.52	0.079	8.48	<10 <sup>-3</sup>	13.39	<10 <sup>-5</sup>	14.88	<10 <sup>-5</sup>	16.88	<10 <sup>-6</sup>	18.66	<10 <sup>-6</sup>
		$(W_0 - W_7) / W_0$		$(W_0 - W_8) / W_0$		$(W_0 - W_9) / W_0$		$(W_0 - W_{10}) / W_0$		$(W_0 - W_{11}) / W_0$			
Covariate	df	F	p	F	p	F	p	F	p	F	p		
SVL	1, 27	9.04	0.006	13.45	0.001	14.92	0.001	14.79	0.001	15.52	0.001		
Species	3, 27	18.32	10 <sup>-6</sup>	21.70	<10 <sup>-7</sup>	22.03	<10 <sup>-7</sup>	21.42	<10 <sup>-7</sup>	20.84	<10 <sup>-7</sup>		
		$(W_0 - W_1) / W_0$		$(W_0 - W_2) / W_0$		$(W_0 - W_3) / W_0$		$(W_0 - W_4) / W_0$		$(W_0 - W_5) / W_0$		$(W_0 - W_6) / W_0$	
Covariates	df	F	p	F	p	F	p	F	p	F	p	F	p
SVL	1, 26	0.56	0.46	0.38	0.54	0.18	0.68	0.01	0.93	0.003	0.96	0.01	0.94
W <sub>0</sub>	1, 26	0.17	0.69	1.03	0.32	2.19	0.15	3.35	0.08	5.02	0.03	4.81	0.04
Species	3, 26	2.27	0.10	7.17	0.001	12.33	<10 <sup>-5</sup>	14.30	<10 <sup>-5</sup>	17.47	<10 <sup>-6</sup>	19.68	<10 <sup>-7</sup>
		$(W_0 - W_7) / W_0$		$(W_0 - W_8) / W_0$		$(W_0 - W_9) / W_0$		$(W_0 - W_{10}) / W_0$		$(W_0 - W_{11}) / W_0$			
Covariates	df	F	p	F	p	F	p	F	p	F	p		
SVL	1, 26	0.16	0.70	0.02	0.90	0.03	0.87	0.01	0.94	0.05	0.83		
W <sub>0</sub>	1, 26	7.05	0.01	8.20	0.01	6.69	0.02	7.33	0.02	6.69	0.02		
Species	3, 26	21.06	<10 <sup>-7</sup>	25.40	<10 <sup>-7</sup>	24.61	<10 <sup>-7</sup>	24.48	<10 <sup>-7</sup>	23.18	<10 <sup>-7</sup>		