Amphibia-Reptilia 30 (2009): 17-23

Preferred temperatures of *Podarcis vaucheri* from Morocco: intraspecific variation and interspecific comparisons

Carla V. Veríssimo[1,2], Miguel A. Carretero[1,*]

Abstract. Preferred body temperatures (Tp) were analysed in the lacertid *Podarcis vaucheri*, from S. Iberia and N. Africa which has been recently revalidated as a full species within the *P. hispanica* complex. Adult lizards from a Moroccan population were exposed to a thermal gradient and Tp was measured at ten time intervals. Intraspecific Tp variation due to sex and reproductive condition (Tp: non-pregnant females $\geqslant$ males $\geqslant$ pregnant females) and time of the day (Tp: early morning > rest of the day) was detected. Pregnant females tended to increase their Tp after egg-laying. This dependence on the reproductive condition parallels findings in other lacertids. Class-by-class comparisons between *P. vaucheri* and *P.* (*hispanica*) *liolepis* from NE Iberia, analysed with the same methodology, revealed higher Tp values in *P. vaucheri* (non-pregnant females). Although further research is needed to determine the evolutionary pattern of the Tp divergence in this group, these results are compatible with the thermal rigidity hypothesis reinforcing the view of *P. hispanica* s.l. as an artificial assemblage.

Keywords: *Podarcis hispanica* complex, *P. vaucheri*, preferred temperatures, pregnancy, thermal ecology.

Introduction

The Iberian and North African members of the lacertid genus *Podarcis* constitute a species complex whose components are gradually being identified on the basis of molecular (Harris and Sá-Sousa, 2001, 2002; Harris et al., 2002; Pinho, Harris and Ferrand, 2003; Pinho, Ferrand and Harris, 2006; Geniez et al., 2007; Pinho, Harris and Ferrand, 2007), morphological (Geniez, 2001; Sá-Sousa, Vicente and Crespo, 2002; Kaliontzopoulou, Carretero and Llorente, 2005; Geniez et al., 2007) and behavioural (Barbosa et al., 2005, 2006) evidence. After the separation of the western Iberian *P. bocagei* (Pérez-Mellado, 1981) and *P. carbonelli* (Sá-Sousa and Harris, 2002), the remaining representatives were tentatively attributed to *P. hispanica** (sensu Harris and Sá-Sousa, 2002). Such an arrangement was, nevertheless, unsatisfactory since this taxon appeared paraphyletic to these two species (Harris and Sá-Sousa, 2002; Pinho, Ferrand and Harris, 2006). Recently, *Podarcis vaucheri* (Boulenger, 1905), from the southernmost Iberia and North Africa, has become the last member of the complex to be recognised at the species level, after its revalidation by Busack, Lawson and Arjo (2005). Whereas other cryptic forms may deserve similar status in the near future (Geniez et al., 2007), comparative studies on their ecology and physiology are urgently needed because the literature is severely biased towards the European forms. Such evidence is necessary not only to assess if previous findings in one of the forms are generalisable from one of the forms to the others (Carretero, Marcos and de Prado, 2006) but may also contribute to understand the evolution of these traits within the group itself.

In this context, preferred body temperature (Tp), constitutes a very relevant ecophysiological trait because it is correlated with several physiological optima directly related with fitness (Huey and Bennet, 1987; Bauwens et al., 1995). In lacertids, Tp changes in response to temporal variation within an individual's life including seasonality, reproductive condition

---

1 - CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

2 - Departamento de Zoologia e Antropologia, Faculdade de Ciências da Universidade do Porto, 4099-002 Porto, Portugal

*Corresponding author; e-mail: carretero@mail.icav.up.pt

© Koninklijke Brill NV, Leiden, 2009. Also available online - www.brill.nl/amre

and feeding status (Castilla, Van Damme and Bauwens, 1999; Carretero, Roig and Llorente, 2005). However, lacertid Tp is evolutionarily rigid (sensu Van Damme, Bauwens and Verheyen, 1990) responding very slowly to long-term changes in the thermal environment. As a consequence, Tp remains similar within species even under different climate regimes (Van Damme et al., 1989; Van Damme, Bauwens and Verheyen, 1990; Tosini and Avery, 1993; Gvozdík and Castilla, 2001; Carretero, Roig and Llorente, 2005), only varying between species (Bauwens et al., 1995). Nevertheless, considerable variation across lizard lineages in the rates of evolutionary response of this trait to directional selection has been detected (Labra, 1998). Consequently, both interspecific and intraspecific variation in Tp must be analysed taking into account the phylogeny and biogeographic history of the groups under question.

The *P. hispanica* complex can be a useful study model for this purpose. In fact, phylogeny and palaeoecological scenarios are now considerably robust (Pinho, Ferrand and Harris, 2006). Marked thermal difference are found in both their current ranges and their putative areas of origin (Sá-Sousa, 2000, 2001; Pinho, Ferrand and Harris, 2006). The Tp of one of the representatives, *P.* (*h.*) *liolepis* from the NE Iberian Peninsula (see Geniez, 2001), has already been analysed (Carretero, Marcos and de Prado, 2006). Although that study revealed patterns of intraspecific variation, doubts persist on the interspecific comparisons with previous studies (Bauwens et al., 1995) because lab methodologies were different. Clearly, populations undoubtedly assigned to one of the forms of the complex must be analysed using the same methodology. If differences between forms were found that would indicate a shift of Tp in the phylogeny. Because such shifts could only affect one sex, reproductive state or activity period, such factors should be taken into account. Because *P. vaucheri* is very distant from *P.* (*h.*) *liolepis* in the most recent phylogeny (Pinho, Ferrand and Harris, 2006) and was originated an environmentally very distinct area (North Africa, according to Pinho, Ferrand and Harris, 2006), it was selected for this preliminary analysis of thermal biology within the group.

Thus, this study analyses variation in selected body temperatures of *P. vaucheri* due to time of day and reproductive condition in order to compare these data with those obtained previously for *P.* (*h.*) *liolepis*.

Material and methods

Twenty seven adult *P. vaucheri* (10 males and 17 females) were collected from Ketama, Rif Massif, Morocco (34°54′N, 4°34′W; 1549 m) in May 2006, coinciding with reproductive peak and oviposition of this species (Schleich, Kästle and Kabisch, 1996). Lizards were kept in individual 0.5 × 0.4 × 0.3 m terraria during not more than two weeks with food (*Acheta domestica* and larval *Tenebrio molitor*) and water provided *ad libitum*. Immediately before each test, the lizards were measured (snout-vent length, SVL) to the nearest 0.05 mm with a digital calliper. Each individual was individually exposed to a photothermal gradient (∼20-45°C, 0.5 × 0.5 × 1.5 m length) produced by a 150-W reflector bulb fixed 15 cm above the substrate maintaining natural photoperiod. The bulb was switched on one hour before the first measurement. Tp was measured by inserting a k-termocouple probe associated with a digital thermometer (HIBOK® 18 data logger thermometer, accuracy 0.01°C) in the cloaca. In order to minimise thermal shifts due to stress or contact with the researcher's hand, no more than 10 seconds elapsed between the capture of the lizard in the terrarium and the temperature measurement. Body temperatures were recorded only for active lizards during a single day at ten consecutive hourly intervals (fig. 1, table 1) distributed throughout the period of diel activity observed in the field (9-18 h, local time, GMT-1). When possible ($n = 7$), females were tested before and after egg-laying in terraria.

Data were not transformed since distributions did not deviate from normality (Shapiro-Wilk test, $p > 0.05$ in all cases), were homoscedastic (univariate Levene tests and multivariate Box M, $p > 0.05$ in all cases) and variances and means were uncorrelated. Since measures were repeated for the same individual (for each interval and, in seven females, before and after egg-laying) statistical analysis was based on Analysis of Variance for Repeated Measures (ANOVAR) of Tp with class (males, pregnant females, non-pregnant females) as between-subject factor and time interval as within-subject factor. Sphericity of the data was assessed by means of Mauchley's tests. Since small lizards could select for lower Tp than large ones due to more opportunistic behaviour (Carretero, Roig and Llorente, 2005) and/or water loss compensation (Bowker, 1993), analyses were repeated using SVL as covariate.

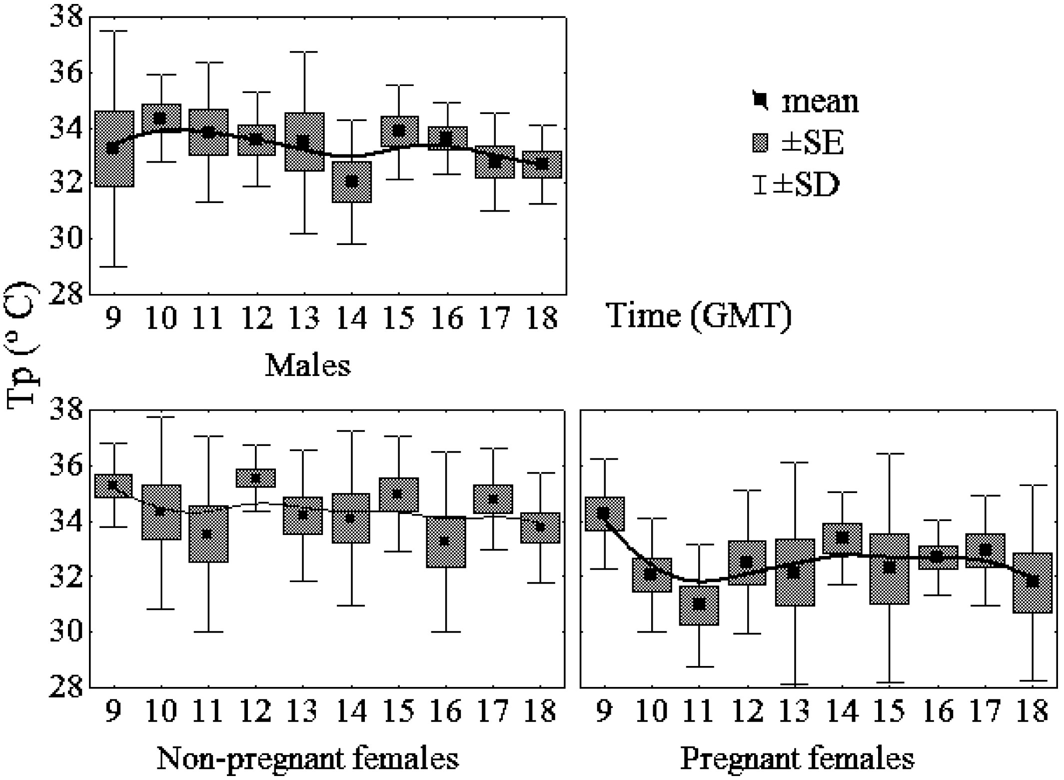

Figure 1. Diel variation of Tp in *P. vaucheri* considering the three classes.

Table 1. Results of the general ANOVAR of Tp considering class and time, and a separate ANOVAR for females considering time.

2-way ANOVAR	F	df	*p*
Class	5.50	2, 30	0.009
Time (R)	1.57	9, 270	0.12
Class × time	1.15	18, 270	0.30
Mauchley's sphericity test	$\chi^2 = 31.31$	44	0.12
Scheffé tests	Non-pregnant females	Pregnant females	
Pregnant females	0.011	–	
Males	0.143	0.533	
2-way ANOVAR (females)	F	df	*p*
Pregnancy (R)	7.10	1, 5	0.04
Time (R)	2.53	9, 45	0.02
Pregnancy × time	0.31	9, 45	0.96

Following Carretero, Roig and Llorente (2005), comparisons between species were not performed on pooled data but class-by-class. Homologous comparisons were performed by means of *t*-tests and corrected by the False Discovery Rate procedure (FDR, Benjamini and Hochberg, 1995). Because methodological issues may pose doubts on the comparativeness of Tp between different studies (Carretero, Roig and Llorente, 2005; Carretero, Marcos and de Prado, 2006), comparisons were restricted to *Podarcis* (*hispanica*) *liolepis* whose Tp were recorded with exactly the same protocol used here (Carretero, Marcos and de Prado, 2006).

Results

Overall Tp (mean±SE, median, range, pregnant females excluded) was 34.00 ± 0.27°C, 34.02, 26.0-37.3. Separating classes, the means of the 10 time intervals averaged 33.43 ± 0.37°C, 33.74, 31.64-34.82 for males; 34.44 ± 0.35, 34.73, 32.07-36.63 for non-pregnant females; and 32.55 ± 0.50, 32.19, 29.58-35.21 for pregnant females. In fact, analyses revealed substantial variation regarding class and, to some extent, time interval (fig. 1). When considering all the individuals, ANOVAR detected significant Tp differences for class but not for time interval (table 1). Scheffé tests indicated that fe-

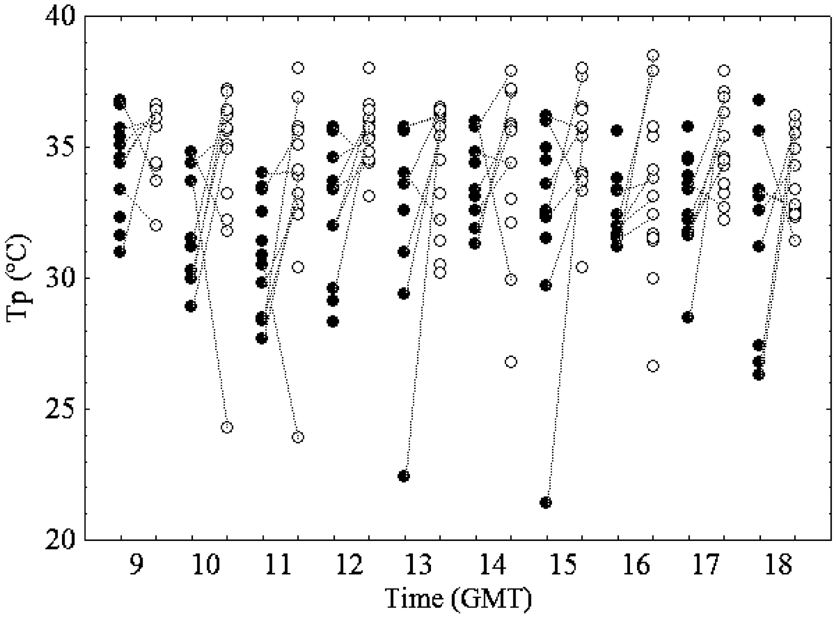

Figure 2. Diel variation of Tp in female *P. vaucheri* showing individual variation after egg-laying. Closed dots: pregnant; open dots: non-pregnant. Dashed lines connect dots belonging to the same individual.

Table 2. Comparative statistics of Tp between two members of the *P. hispanica* complex: *P.* (*h.*) *liolepis* (after Carretero et al., 2006) and *P. vaucheri* (this study). Pooled calculations excluded pregnant females. * also significant after FDR correction.

Class	*P. vaucheri* (Morocco)		*P.* (*h.*) *liolepis* (NE Iberia)		Comparison		
	n	mean ± SE	*n*	mean ± SE	*T*	df	*P*
Males	10	33.43 ± 0.37	9	33.75 ± 0.24	0.70	17	0.49
Non preg. females	13	34.44 ± 0.35	7	32.20 ± 0.31	4.25	18	0.0005*
Pooled	23	34.00 ± 0.27	16	33.07 ± 0.27	2.34	37	0.0246*
Preg. females	11	32.55 ± 0.50	2	33.18 ± 0.06	0.52	11	0.62

males attained lower Tp values when pregnant but failed to detect differences between males and females, either pregnant or non-pregnant (table 1). When a separate analysis for females was conducted, both class and time were significant (table 1). A clear decrease in Tp with pregnancy was confirmed and, in fact, individual females tracked before and after egg-laying tended to display systematic Tp increases (fig. 2). Besides that, lizards, at least females, also selected for higher temperatures in the early morning (fig. 1). Although no correlation between mean Tp and body size was detected in any case ($p > 0.05$), analyses were repeated using SVL as covariate and results were identical.

Comparisons of these results with those already published for *P.* (*h.*) *liolepis* (table 2) indicate overall differences between both forms, with *P. vaucheri* selecting higher temperatures. However, when analysing each class separately, such differences only persisted for non-pregnant females whereas males and pregnant females were not found to be significantly distinct.

Discussion

As in other lacertid lizards, *Podarcis vaucheri* keeps its body temperature within a narrow range when free of thermal constraints (see Castilla, Van Damme and Bauwens, 1999 and references therein). This thermoregulatory set point can, nevertheless, be modified according to the individual's traits such as sex, nutritional state, reproductive condition (season, pregnancy) and activity time (Castilla, Van Damme and Bauwens, 1999; Gvozdík and Castilla, 2001; Carretero, Roig and Llorente, 2005). Because the whole study was carried out during a short period of time and animals were in good nutritional state (Brown and Griffin, 2003), sex, pregnancy and diel activity are the factors explored here.

Pregnant females of *Zootoca vivipara* tended to adjust their Tps according to the embryo requirements rather than to themselves (Van Damme, Bauwens and Verheyen, 1986; Gvozdík and Castilla, 2001) and, subsequently, changed them back after parturition or egg laying to approach values found in males (Carretero, Roig and Llorente, 2005). Results obtained here together with those for *P. muralis* by Tosini and Avery (1996) indicate that this can be also the case for *Podarcis* and probably for other lacertids even with short gestation periods. Whether pregnancy systematically promotes a decrease in Tp, needs further comparative research. Previous suggestions that Tp may be lower after pregnancy in *P.* (*h.*) *liolepis* (Carretero, Marcos and de Prado, 2006) are preliminary and not supported by sufficient sample size ($n = 2$).

Regarding the diel pattern observed, it appears to be weaker but consistent with that reported for other species (Rismiller and Heldmaier, 1982; Tosini and Avery, 1996; Carretero, Roig and Llorente, 2005; Carretero, Marcos and de Prado, 2006). Lacertids tend to raise Tp in the early morning when environmental temperatures are low and thermoregulation becomes a priority in relation to other requirements; the same does not apply at the end of the day (Carretero, Marcos and de Prado, 2006). Results were masked when comparing all three classes but were significant when males were excluded, with females displaying the highest shift. It is worth noting that the temperatures attained by pregnant females were closer to those of non-pregnant females in the early morning whereas clearly differed from them during the rest of the day. This finding suggest that, at least during this period, the mother's thermoregulation may have precedence.

As previously commented, in order to assess whether *P. vaucheri* displays different Tp from *P. hispanica* s.l., a careful methodological approach was followed. Comparisons were conducted only with *P.* (*h.*) *liolepis*, analysed with the same laboratory methods, and between homologous classes. Actually, although overall comparison suggested that *P. vaucheri* selects for higher Tp, in fact, this was restricted to non-pregnant females whereas the analyses failed to detect differences in males, the class less affected by changes in body condition (Bauwens et al., 1995). This provides only limited support for a thermal shift between both forms.

According to their genetic distances, *P. vaucheri* and *P.* (*h.*) *liolepis* are among the most distant members of the *P. hispanica* complex and belong to quite divergent clades within the phylogeny (Pinho, Ferrand and Harris, 2006). In fact, molecular clock estimates suggest that both forms have undergone up to 10 my of separate evolution (Pinho, Ferrand and Harris, 2006), that is, clearly predating the strong thermal changes of the Plio-Pleistocene. Furthermore, it is expected that their distributional ranges have been differentially affected by the glaciations (Pinho, Ferrand and Harris, 2006), with *P. vaucheri* in North Africa having faced milder conditions than *P.* (*h.*) *liolepis* in NE Iberia during the glacial maxima. Consequently, long evolutionary time and divergent (but relatively recent) thermal conditions could have selected for evolutionary shifts in Tp.

In conclusion, at an intraspecific level, results parallel variation found in other lacertid species whereas, at an interspecific level, differences found are compatible with the thermal rigidity hypothesis (Van Damme et al., 1990) reinforcing the view of *P. hispanica* s.l. as an artificial assemblage (Pinho, Ferrand and Harris, 2006). However, further research on others members of the complex is still needed to elucidate which is the evolutionary tempo of the Tp divergence in this group, which are the palaeoecological scenarios involved and what is the impact of homoplasy on this ecophysiological trait.

Acknowledgements. The authors are indebted to M.J. Amaral, A. Kaliontzopoulou and R. Ribeiro, as well as to all the CIBIO members of who helped in lizard collection. This research was funded by the projects POCI/BIA-BDE/55865/2004 and POCI/BIA-BDE/56931/2004 and M.A.C. held the post-doctoral contract SFRH/BPD/27025/2006, all from Fundação para a Ciência e a Tecnologia, FCT (Portugal).

References

Barbosa, D., Desfilis, E., Carretero, M.A., Font, E. (2005): Chemical stimuli mediate species recognition in *Podarcis* wall lizards. Amphibia-Reptilia **26**: 257-263.

Barbosa, D., Font, E., Desfilis, E., Carretero, M.A. (2006): Sex differences in species discrimination between closely related *Podarcis* wall lizards. J. Chem. Ecol. **32**: 1587-1598.

Bauwens, D., Garland, T., Jr., Castilla, A.M., Van Damme, R. (1995): Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioural covariation. Evolution **49**: 848-863.

Benjamini, Y., Hochberg, Y. (1995): Controlling the false discovery rate: A practical and powerful approach to multiple testing. J. R. Statist. Soc. B **57**: 289-300.

Bowker, R.G. (1993): The thermoregulation of the lizards *Cnemidophorus exanguis* and *C. velox*: some consequences of high body temperature. In: Biology of Whiptail Lizards (genus *Cnemidophorus*), p. 117-132. Wrigth, J.W., Vitt, L.J., Eds, Norman, Oklahoma Mus. Nat. Hist.

Brown, R.P., Griffin, S. (2003): Lower selected body temperatures after food deprivation in the lizard *Anolis carolinensis*. J. Therm. Biol. **30**: 79-83.

Busack, S.D., Lawson, D.P., Arjo, W.M. (2005): Mitochondrial DNA, allozymes, morphology and historical biogeography in the *Podarcis vaucheri* (Lacertidae) species complex. Amphibia-Reptilia **26**: 239-256.

Carretero, M.A., Marcos, E., de Prado, P. (2006): Intraspecific variation of preferred temperatures in the NE form of *Podarcis hispanica*. In: Mainland and Insular Lacertid Lizards: A Mediterranean Perspective, p. 55-64. Corti, C., Lo Cascio, P., Biaggini, M., Eds, Florence, Firenze University Press.

Carretero, M.A., Roig, J.M., Llorente, G.A. (2005): Variation in preferred body temperature in an oviparous population of *Lacerta* (*Zootoca*) *vivipara*. Herpetol. J. **15**: 51-55.

Castilla, A.M., Van Damme, R., Bauwens, D. (1999): Field body temperatures, mechanisms of thermoregulation, and evolution of thermal characteristics in lacertid lizards. Natura Croatica **8**: 253-274.

Geniez, P. (2001): Variation géographique des lézards du genre *Podarcis* (Reptilia, Sauria, Lacertidae). Thesis Ecole Practique des Hautes Etudes, Montpellier.

Geniez, P., Cluchier, A., Sá-Sousa, P., Guillaume, C.P., Crochet, P.A. (2006): Systematics of the *Podarcis hispanicus*-complex (Sauria, Lacertidae): I – Redefinition, morphology and distribution of the nominotypical taxon. Herpetol. J. **17**: 69-80.

Gvozdík, L., Castilla, A.M. (2001): A comparative study of preferred body temperatures and critical thermal tolerance limits among populations of *Zootoca vivipara* (Squamata: Lacertidae) along an altitudinal gradient in the Czech Republic. J. Herpetol. **35**: 486-492.

Harris, D.J., Carranza, S., Arnold, E.N., Pinho, C., Ferrand, N. (2002): Complex biogeographical distribution of genetic variation within *Podarcis* wall lizards across the Strait of Gibraltar. J. Biogeogr. **29**: 1257-1262.

Harris, D.J., Sá-Sousa, P. (2001): Species distinction and relationships of the western Iberian *Podarcis* lizards (Reptilia, Lacertidae) based on morphology and mitochondrial DNA sequences. Herpetol. J. **11**: 129-136.

Harris, D.J., Sá-Sousa, P. (2002): Molecular phylogenetics of Iberian wall lizards (*Podarcis*): is *Podarcis hispanica* a species complex? Mol. Phylogenet. Evol. **23**: 75-81.

Huey, R.B., Bennett, A.F. (1987): Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. Evolution **41**: 1098-1115.

Kaliontzopoulou, A., Carretero, M.A., Llorente, G.A. (2005): Differences in the pholidotic patterns of *Podarcis bocagei* and *P. carbonelli* and implications for species determination. Rev. Esp. Herpetol. **19**: 71-86.

Labra, A. (1998): Selected body temperatures of seven species of Chilean *Liolaemus* lizards. Rev. Chil. Hist. Nat. **71**: 349-358.

Pérez-Mellado, V. (1981): La lagartija de Bocage *Podarcis bocagei* (Seoane, 1884): Primeros datos sobre su distribución, colorido y ecología. Amphibia-Reptilia **3/4**: 253-268.

Pinho, C., Ferrand, N., Harris, D.J. (2006): Reexamination of the Iberian and North African *Podarcis* (Squamata: Lacertidae) phylogeny based on increased mitochondrial DNA sequencing. Mol. Phylogenet. Evol. **38**: 266-273.

Pinho, C., Harris, D.J., Ferrand, N. (2003): Genetic polymorphism of 11 Allozyme Loci in populations of wall lizards (*Podarcis* sp.) from the Iberian Peninsula and North Africa. Biochem. Genet. **41**: 343-359.

Pinho, C., Harris, D.J., Ferrand, N. (2007): Contrasting patterns of population subdivision and historical demography in three western Mediterranean lizard species inferred from mitochondrial DNA variation. Mol. Ecol. **16**: 1191-1205.

Rismiller, P.D., Heldmaier, G. (1982): The effect of photoperiod on temperature selection in the European green lizard, *Lacerta viridis*. Oecologia (Berl.) **53**: 222-226.

Sá-Sousa, P. (2000): A predictive distribution model for the Iberian wall lizard (*Podarcis hispanicus*) in Portugal. Herpetol. J. **10**: 1-11.

Sá-Sousa, P. (2001): Comparative chorology between *Podarcis bocagei* and *P. carbonelli* (Sauria: Lacertidae) in Portugal. Rev. Esp. Herpetol. **15**: 85-97.

Sá-Sousa, P., Harris, D.J. (2002): *Podarcis carbonelli* Pérez-Mellado, 1981 is a distinct species. Amphibia-Reptilia **23**: 459-468.

Sá-Sousa, P., Vicente, L., Crespo, E.G. (2002): Morphological variability of *Podarcis hispanica* (Sauria: Lacertidae) in Portugal. Amphibia-Reptilia **23**: 55-69.

Schleich, H.H., Kästle, W., Kabisch, K. (1996): Amphibians and Reptiles of North Africa. Koeltz. Koenigstein, Germany.

Tosini, G., Avery, R.A. (1993): Intraspecific variation in lizard thermoregulatory set points: a thermographic study in *Podarcis muralis*. J. Therm. Biol. **18**: 19-23.

Tosini, G., Avery, R.A. (1996): Pregnancy decreases set point temperatures for behavioural thermoregulation in the wall lizard *Podarcis muralis*. Herpetol. J. **6**: 84-96.

Van Damme, R., Bauwens, D., Castilla, A.M., Verheyen, R.F. (1989): Altitudinal variation of thermal biology and running performance in the lizard *Podarcis tiliguerta*. Oecologia (Berl.) **80**: 516-524.

Van Damme, R., Bauwens, D., Verheyen, R.F. (1986): Selected body temperatures in the lizard *Lacerta vivipara*: variation within and between populations. J. Therm. Biol. **11**: 219-222.

Van Damme, R., Bauwens, D., Verheyen, R. (1990): Evolutionary rigidity of thermal physiology: the case of the cool temperate lizard *Lacerta vivipara*. Oikos **57**: 61-67.

Received: January 3, 2006. Accepted: July 16, 2008.