



Separate histories in both sides of the Mediterranean: phylogeny and niche evolution of ocellated lizards

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ABSTRACT

Aim The ocellated lizards of the genus *Timon*, comprising six species that are distributed across continental Asia, Europe and Africa, offer an interesting model to study the evolution of ecological niches through comparative phyloclimatic analysis. Our study provides insights into the evolutionary history of the ocellated lizards and helps to understand the role of climatic niche evolution during the speciation process.

Location Eastern and western margins of the Mediterranean basin.

Methods A dated molecular phylogeny was estimated based on three mitochondrial and two nuclear genes. Using multivariate statistics, species distribution models were developed to characterize the Grinnellian niches of all species. Subsequently, ancestral environmental niche occupancy of each taxon was reconstructed using niche occupancy profiles. Niche divergence among species was quantified by computing multivariate niche overlaps via two-dimensional and *n*-dimensional approaches.

Results Phylogenetic analysis supports that the ancestor of *Timon* diverged into the eastern and western groups following multiple vicariance events that shaped the current distribution pattern of Palaearctic lizards. High complexity in the ecological niche evolution between the Mediterranean and non-Mediterranean climatic regions was detected. The generally low niche divergence among members of the eastern group and the remarkable climatic divergence within the western group highlight an important role of temperature seasonality in a Mediterranean and Atlantic climate context. The results also suggest niche conservatism in terms of microhabitats described by vegetation cover.

Main conclusions The ocellated lizards provide an interesting example of a vertebrate radiation where niche shift (with or without vicariance) and niche conservatism alternate in different niche axes shaping current biogeographical patterns.

Keywords

ancestral niche reconstruction, *n*-dimensional hypervolume, niche overlap, species distribution modelling, *Timon*, vicariance

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INTRODUCTION

The multidisciplinary approach required to characterize and describe the ecological niche of species has led to numerous niche concepts. According to the Grinnellian niche concept (Grinnell, 1917), a species' niche is determined by the sum of environmental variables within the species' habitat (i.e.

scenopoetic variables), allowing a population to persist infinitely. Hutchinson (1958), elaborating on the Grinnellian niche concept, proposed that a species' niche could be defined as an imaginary multidimensional hyperspace, in which each dimension represents an environmental condition or resource that is required to sustain a viable population.

The spatial and temporal distribution of a species is shaped by its fundamental niche, which determines the potential large-scale distribution. Taking into account its biotic interactions, its realized niche constitutes the parts of its fundamental niche that are actually represented in geographical space (see also Soberón, 2007). Assessments of how the fundamental and realized niches of closely related species have evolved through time can help to explain observed biogeographical patterns by testing explicit ecological hypotheses (i.e. whether patterns of niche conservatism or divergence dominate). Not surprisingly, the integration of niche evolution as a useful tool in historical biogeography and evolutionary research has increased in popularity in recent years (Rice *et al.*, 2003; Graham *et al.*, 2004; Evans *et al.*, 2009; Jakob *et al.*, 2010; Ahmadzadeh *et al.*, 2013; Rato *et al.*, 2015).

Incorporating environmental and ecological data in a phylogenetic context allows the identification of phylogenetic signals in niche axes. These analyses provide a measure of niche similarity between related species, which can be used to build hypotheses on niche and range evolution. In turn, this allows quantification to what degree biodiversity patterns may be a result of niche conservatism, a topic which has received much attention in recent biogeographical literature (Nyári & Reddy, 2013). Several studies comparing niche traits of closely related species within a phylogenetic framework and/or among native and alien populations revealed some degree of niche conservatism (Graham *et al.*, 2004; Wiens & Graham, 2005; Broennimann *et al.*, 2007; Peterson & Nyari, 2007), although these findings are not free of dispute (e.g. Pearman *et al.*, 2008; Ahmadzadeh *et al.*, 2013).

With six species distributed across continental Asia, Europe and Africa (Fig. 1), the ocellated lizards of the genus *Timon* represent an interesting model to study the evolution of ecological niches through comparative phloclimatic analysis. This monophyletic genus (Pyron *et al.*, 2013) can be divided into two geographical clades. *Timon kurdistanicus* (Suchow, 1936), recently elevated to species level (Ahmadzadeh *et al.*, 2012), and *Timon princeps* (Blanford, 1874) constitute the eastern clade distributed in Turkey, northern Iraq and the Zagros mountains in Iran (Eiselt, 1968; Anderson, 1999; Ilgaz & Kumlutás, 2008; Ahmadzadeh *et al.*, 2012). The western clade consists of four species, with *Timon pater* (Lataste, 1880) and *Timon tangitanus* (Boulenger, 1881) inhabiting the Mediterranean region and the Atlas mountain range in north-western Africa (Paulo *et al.*, 2008; Perera & Harris, 2010), and *Timon lepidus* (Daudin, 1802) and *Timon nevadensis* (Buchholz, 1963) occurring in south-western Europe (Miraldo *et al.*, 2012). The latter two species form the only documented case of parapatry in the genus, with a reported narrow contact zone in south-eastern Iberia (Miraldo *et al.*, 2011, 2012; Nunes *et al.*, 2011a). In general, ocellated lizards inhabit mesic habitats covered with bushy vegetation where large rocks are frequently available. However, the environments occupied by the eastern and western clades differ. The western group is exposed to a wider range

of climatic conditions, with members occurring in both Mediterranean and Atlantic regions (Arnold *et al.*, 2007). Tolerated climatic conditions also vary among members of the eastern group, ranging from thermo-Mediterranean climate in Turkey to xero-thermo-Mediterranean climate in the southern Zagros Mountains, which are characterized by dry summers and cold winters (Eiselt, 1968; Anderson, 1999). These marked differences in the climatic conditions that species of the ocellated lizards are exposed to might have played an important role during the speciation process. The potentially important role of the environment in shaping the speciation process in these lizards has been recently highlighted by Nunes *et al.* (2011a,b), who provide the first assessment of the correlation between environmental factors and ecologically relevant phenotypes in *T. lepidus* and *T. nevadensis*. However, it remains unclear how environmental variables have influenced the biogeography and evolutionary processes in the ocellated lizards.

In this study, we infer the phylogenetic relationships and divergence times among species of *Timon* and use spatial environmental variables based on newly computed satellite derived data in order to (1) quantify the niche overlap among all *Timon* taxa, (2) predict the potential distribution of each species and (3) estimate their niche evolution by using a combination of dated molecular phylogenetic information and georeferenced occurrence records to investigate if niche conservatism or niche shift have contributed to the biogeography of the species. We hypothesize that initial niche divergences between ancestral clades of *Timon* evolved due to allopatry, but later were complemented by niche shifts. Our analyses aim to provide insights into the evolutionary history of the ocellated lizards and help understanding the role of climatic niche evolution during the speciation process in this group of lizards.

MATERIALS AND METHODS

Taxon sampling, molecular phylogenetic analyses and estimation of divergence times

Sampling encompasses 36 specimens covering all six species of the genus *Timon*. In addition, three samples of *Lacerta*, representing the sister genus of *Timon* (Pyron *et al.*, 2013), were implemented as outgroup. Genes, accession numbers and respective localities of specimens used in this study are provided in Supporting Information Appendix S1. Total genomic DNA from each individual was extracted using standard saline methods (Sambrook *et al.*, 1989) and three mitochondrial genes (cytochrome *b*, 16S rRNA and 12S rRNA) and two nuclear genes (*c-mos* and β -fibrinogen) were amplified. Primers used, PCR cycling conditions and sequencing protocol of the purified products are described in Ahmadzadeh *et al.* (2012). Chromatographs of each sequence were checked using the program CodonCode (CodonCode Corporation, Dedham, MA, USA) and sequences were subsequently aligned with MUSCLE 3.8 (Edgar, 2004). The

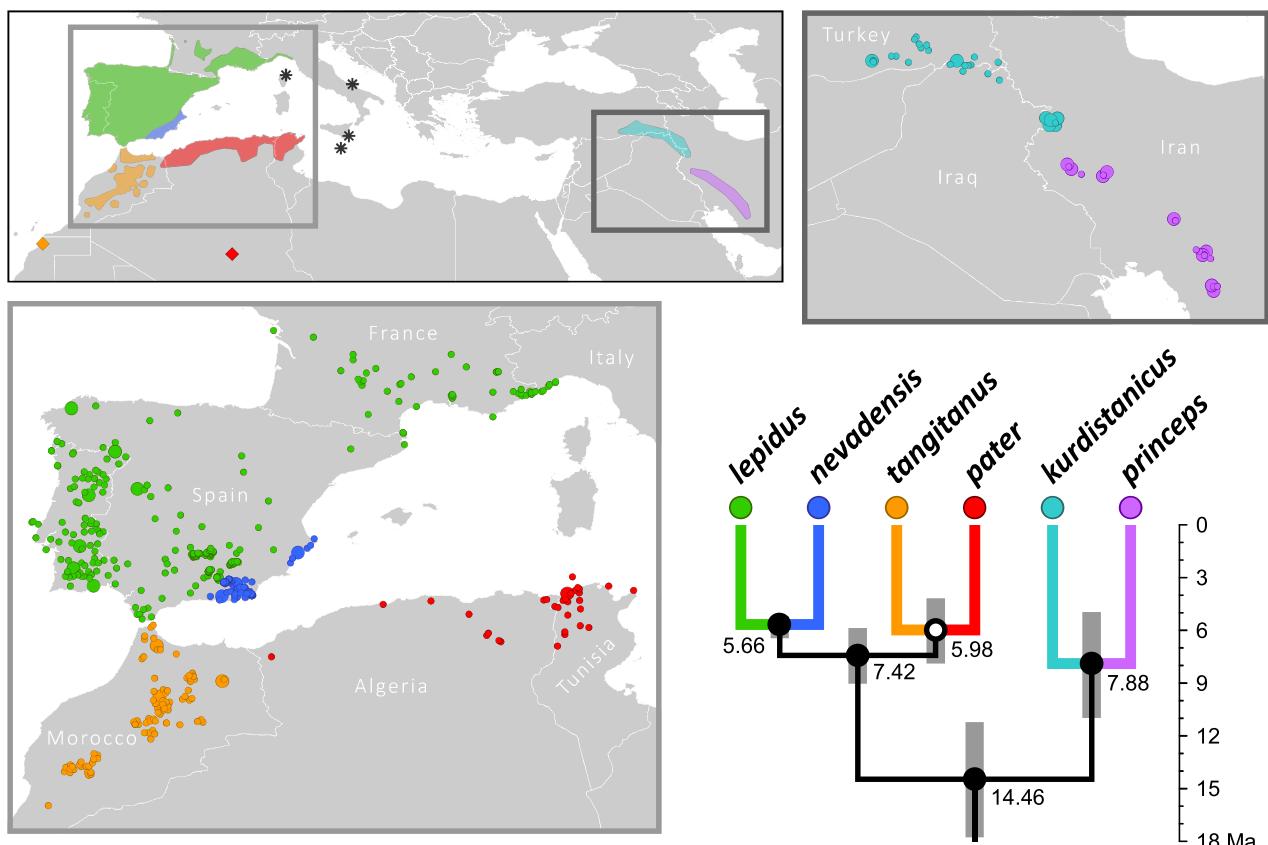


Figure 1 Current distribution ranges of each *Timon* species (shaded areas) and the two known relict populations (marked by diamonds). Asterisks mark Pleistocene fossil records outside of the genus' current range. Points in detail maps represent localities that were used for species distribution modelling, larger symbols represent populations also included in the genetic sampling. The dated maximum clade credibility species tree (inferred with *BEAST based on multi-locus data) shows the phylogenetic relationships and estimated divergence dates within *Timon* (outgroup not shown for clarity). Nodes with a posterior probability ≥ 0.95 are marked by solid black circles. Grey bars show 95% highest posterior density intervals of estimated node ages and values on nodes are mean node ages (in Ma).

alignment was manually refined where necessary. Models of nucleotide substitution were chosen using the Akaike's information criterion in MrMODELTEST 2.3 (Nylander, 2004). The concatenated multi-locus data set contains 2766 base pairs (bp). Detailed properties and chosen models are shown in Appendix S1.

Phylogenetic relationships and divergence times within *Timon* were estimated with *BEAST 1.7.5 (Heled & Drummond, 2010). *BEAST produces estimates of the species tree (which we used as an input for calculating the niche occupancy profiles, see below) by calculating the multispecies coalescent with Bayesian Markov Chain Monte Carlo algorithm (MCMC). We used a relaxed molecular clock approach with an estimated clock rate and uncorrelated log-normal distribution (Drummond *et al.*, 2006). The multi-locus data set was partitioned by gene and codon position with model parameters estimated separately for each partition. The Yule speciation process was set as species tree prior and a starting tree (topology inferred with the same settings in *BEAST, but without the constraints on divergence time) was provided. Two fossils were used for calibration: *Lacerta*

ruscinensis (5.3 Ma, indistinguishable from modern *T. lepidus*; Depéret, 1890; Hipsley *et al.*, 2009) for the split of *T. lepidus* and *T. nevadensis* and *Lacerta* sp. (17.5 Ma, different from *T. lepidus*, but indistinguishable from *Lacerta* s. str.; Cernansky, 2010) for the split of *Timon* and *Lacerta*. These calibration points were implemented as single-sided hard bounds (i.e. with a fixed lower bound and a gamma prior distribution). Results of the *BEAST analyses were summarized from four independent runs with 10^8 generations each, sampling every 10^4 trees and omitting the initial 10% as burn-in after checking for convergence and sufficient effective sample sizes ($ESS > 500$) with TRACER 1.5 (Rambaut & Drummond, 2007).

Occurrence records and environmental data

A total of 568 species occurrence records ranging from 22 for *T. princeps* to 299 for *T. lepidus* were obtained from own and other herpetologists' field work, scientific publications, and scientific collections (see Table S2.1 in Appendix S2). The locality data were georeferenced using the global

gazetteer version 2.1 (<http://www.fallingrain.com/world>). Spatial accuracy of the coordinates was checked by mapping them in DIVA-GIS 7.5 (Hijmans *et al.*, 2005, available through <http://www.diva-gis.org>). Records with a coordinate uncertainty larger than the spatial or temporal resolution of the environmental variables (see below) were dismissed. The range of the occurrence records reflects the general range size of the species. The species records were subsequently resampled to a minimum distance of 15 km between records to remove potential negative effects of spatial autocorrelation, which was assessed using a semivariogram based on Moran's *I*.

In order to capture the realized environmental space of the taxa, we used a set of multi temporal remote-sensing variables derived from pre-processed monthly variables over the time period 2001–2005 from MODIS sensors of two NASA satellites with a spatial resolution of 30 arc seconds. The monthly variables were provided by the EDENext project (www.edenext.eu; data set MODIS v4). These include middle infra-red (MIR; quantifying the water content of the surface; Jensen, 2007), daytime and night time land surface temperature, normalized difference vegetation index (NDVI; Tucker, 1979), and enhanced vegetation index (EVI; Huete *et al.*, 1997; for details see Mu *et al.*, 2007 and Scharlemann *et al.*, 2008). Annual and seasonal variation was captured using the DISMO and RASTER packages (Hijmans & Etten, 2012; Hijmans *et al.*, 2012) for CRAN R (R Core Team, 2015). Variables and meaning of derived variables are provided in the Appendix S2 (see Table S2.2). As a general strategy we used temporal snapshots analogue to the original bioclim variables to capture seasonal temperature, MIR and NDVI changes, e.g. for NDVI we computed analogue to bio10 and bio11, the most productive and least productive vegetation seasons, as consecutive three months to estimate seasonal changes in mean productivity. The complete set of variables describes the seasonal changes in the habitat of the species in terms of temperature, humidity and vegetation structure.

To estimate the environmental space available to each taxon, we randomly selected 1000 occurrence records covering the species' entire range as estimated by a minimum convex polygon enclosing the pooled set of species records. Based on these random points, a principal component analysis (PCA) was performed to eliminate multi-collinearity of predictors and principal components (PCs) with an eigenvalue exceeding 1 were projected back into geographical space (see Table S2.2 in Appendix S2). A PCA trained with random records allowed us to capture the general environmental properties of the study area and has the advantage that it is less sensitive to the relative proportions of records available for each species.

Niche comparisons

Hutchinson's hypervolume can be described by a set of species records within *n*-dimensional space that reflects environmentally suitable habitats (Hutchinson, 1957). This is a

fundamental concept in ecology and can be used to quantify the niche of a species. For univariate comparisons of environmental niche overlaps among taxa across the four principal components were visualized using density profiles computed using the *sm.density.compare* function of the *sm* package (Bowman & Azzalini, 1997) for CRAN R. As second and third approaches, the multivariate environmental niche overlaps between the extant species of *Timon* were quantified following both the 'PCA-env' approach as proposed by Broennimann *et al.* (2012) and the direct estimation of the *n*-dimensional hypervolume from a set of observations (Blonder *et al.*, 2014; see below) respectively.

The 'PCA-env' approach has recently become widely used to measure inter- as well as intraspecific ecological niche overlap based on occurrence data sets and spatial environmental data (Broennimann *et al.*, 2012). Niche overlap within the first two principal components in PCA space is quantified by means of Schoener's (1970) *D* index accounting for the frequency of specific environmental conditions within the available niche space of the species. Using null models, niche equivalency and similarity are assessed following Warren *et al.* (2008) as modified by Broennimann *et al.* (2012).

Because of the poor ability of current methods to sample from the *n*-dimensional hypervolume, a new approach was recently introduced by Blonder *et al.* (2014), in which its geometry and topology can be measured. The technique is based on a new multivariate kernel density estimation method in an arbitrary number of dimensions (Blonder *et al.*, 2014). We applied this novel approach to delineate the four dimensional environmental hypervolumes of each species pair in terms of shape and volume following two procedures: (1) using a bandwidth of 0.5 enclosing species records as originally suggested by Blonder *et al.* (2014) and (2) applying a multivariate minimum convex polygon (MMCP) approach as recently introduced in the HYPERVOLUME package (Blonder *et al.*, 2014) for CRAN R. Then, the total volume (union of both hypervolumes), the overlap (intersection) between two hypervolumes and the unique part of each hypervolume were determined. Subsequently, the environmental hypervolume for each species was projected into geographical space. To determine the predictive performance of the hypervolume SDMs, species records were randomly subdivided ten times into 70% used for model training while the remaining 30% were used to test the model using the area under the 'receiver operating characteristic curve' (AUC; Swets, 1988) and the 'point biserial correlation' (COR; Elith & Graham, 2006) as quality metrics. Average values across all 10 replicates were used for further processing. All calculations were conducted using the HYPERVOLUME and DISMO packages in CRAN R 3.0.0 as well as customized scripts provided by Broennimann *et al.* (2012).

Niche occupancy profiles

We used the ancestral niche occupancy reconstruction approach proposed by Evans *et al.* (2009), which facilitates

the quantification of differences among phylogenetic clades regarding their occupied environmental niches as well as the reconstruction of temporal development of niche disparity. Each species' potential distribution, as suggested by the hypervolume SDM, was combined with the dated, ultrametric species tree to investigate the environmental niche evolution among species within the genus *Timon* through predicted niche occupancy (PNO) profiles (Evans *et al.*, 2009; Jakob *et al.*, 2010; Ahmadzadeh *et al.*, 2013). To reconstruct ancestral environmental niche occupancy profiles, each species' occurrence probability at a given grid cell in the study area, as suggested by the SDM, was combined with the corresponding scores of the original PCs, creating a vector of probabilities per binned PC scores (100 intervals herein). The PNO assigns a probability of suitability for the target species across the range of the PC, taking the frequency of specific conditions within the geographical space into account. The ancestral environmental tolerance for each PC was estimated from the PNO profiles under the assumption of Brownian motion evolution for each node, wherein the PNO profiles are resampled for 1000 times and the ancestral character reconstructions are performed using generalized least squares (see Evans *et al.*, 2009; Ahmadzadeh *et al.*, 2013). All computations were conducted using the PHYLOClim package (Heibl & Calenge, 2013) for CRAN R 3.0.0.

RESULTS

Species tree and divergence times

The two sister genera *Lacerta* and *Timon* are estimated to have diverged 18.6 Ma (95% highest posterior density (HPD) interval: 17.5 Ma–20.6 Ma), with the latter forming a strongly supported monophyletic group. *Timon* consists of two main clades, which diverged 14.5 Ma (HPD: 11.2 Ma–17.8 Ma) and are in accordance with its disjunct distribution (Fig. 1). The eastern clade includes the two oriental species *T. princeps* and *T. kurdistanicus*, which split 7.9 Ma (HPD: 5.0 Ma–11.0 Ma). The western clade consists of the European subclade with the species *T. lepidus* and *T. nevadensis*, and the African subclade with the species *T. pater* and *T. tangitanus*. These two subclades are estimated to have split 7.4 Ma (HPD: 5.9 Ma–9.0 Ma), while species inside the European and African subclades have split 5.7 Ma (HPD: 5.3 Ma–6.3 Ma) and 6.0 Ma (HPD: 4.2 Ma–7.9 Ma) respectively.

Species distribution models, niche overlap and niche occupancy profiles

The contribution of each of the 30 original environmental variables to each PC is shown in Table S2.2 in Appendix S2. The first and second principal components (PC1: eigenvalue 17.91; PC2: eigenvalue 7.01) explained 59.70 and 23.39% of the total variation among all 30 environmental variables respectively. The annual mean of the Middle-Infra-Red

(ED1503_bio1) has the highest contribution to PC1 followed by the maximum monthly Normalized Difference Vegetation Index (ED1514_bio5). PC2 is mainly characterized by temperature seasonality (ED150708_bio4) and Middle Infra-Red seasonality (ED1503_bio4). The annual range of Middle Infra-Red (ED1503_bio7) and seasonality of Middle Infra-Red (ED1503_bio4) contributed most to PC3. PC4 summarizes the seasonality of the Normalized Difference Vegetation Index (ED1514_bio4) and the annual range of the Normalized Difference vegetation Index (ED1514_bio7).

Using the bandwidth approach (a) the average AUC values for the hypervolume SDMs of the six species of *Timon* range from 0.764 in *T. lepidus* to 0.935 in *T. pater*. For the other species the AUC scores are: *T. tangitanus* (0.809), *T. nevadensis* (0.878), *T. princeps* (0.910) and *T. kurdistanicus* (0.914). Applying the (MMCP) method the AUC scores were generally similar (*T. tangitanus* 0.837, *T. pater* 0.833, *T. nevadensis* 0.808, *T. lepidus* 0.797, *T. kurdistanicus* 0.765 and *T. princeps* 0.676). According to previous classifications (Swets, 1988; Araujo & New, 2007), our results indicate a good discrimination ability of the SDMs for most of the cases, although SDMs for those species occupying large ranges (e.g. *T. lepidus*) had lower discrimination ability, as expected. COR scores were highest in western species, *T. nevadensis* (bandwidth: 0.457; MMCP: 0.502), *T. lepidus* (0.452; 0.504), *T. pater* (0.409; 0.377), *T. tangitanus* (0.349; 0.491) and lowest in eastern species, *T. princeps* (0.298; 0.368) and *T. kurdistanicus* (0.340; 0.373). As the results obtained from both approaches were very similar, further analyses were restricted to the bandwidth approach.

Although the potential distributions suggested by the hypervolumes (Fig. 2) overlap in some regions, they are generally in concordance with presently known distribution ranges (Fig. 1). It was found that *T. lepidus* has the largest volume in concordance with the largest geographical range. The smallest volume and distribution range is occupied by *T. princeps*. However, the *n*-dimensional hypervolume approach also captures areas that are not included in the currently realized geographical distributions of the species, indicating extensive overlaps.

Results of the multivariate niche overlap analyses are presented in Table 1 and Fig. 3 (see Appendix S3 for detailed results and plots). The well-known low-dimensional system (e.g. Broennimann *et al.*, 2012) is not computationally practicable on high dimensional or disjunct data sets. However, direct estimation of the *n*-dimensional hypervolume from a set of observations is successfully achieved via the new method (Blonder *et al.*, 2014). Regarding the 'PCA-env' approach, niche overlap between species-pairs in terms of Schoener's *D* ranges from 0.01 (*T. pater* and *T. princeps*) to 0.52 (*T. pater* and *T. tangitanus*). When comparing sister species, *T. lepidus* and *T. nevadensis* have the lowest overlap (*D* = 0.20), which is exceeded by the overlaps of *T. lepidus* with each of the less closely related species of the western clade (i.e. the two African species). Niche equivalency tests were rejected for all pairwise comparisons ($P < 0.01$),

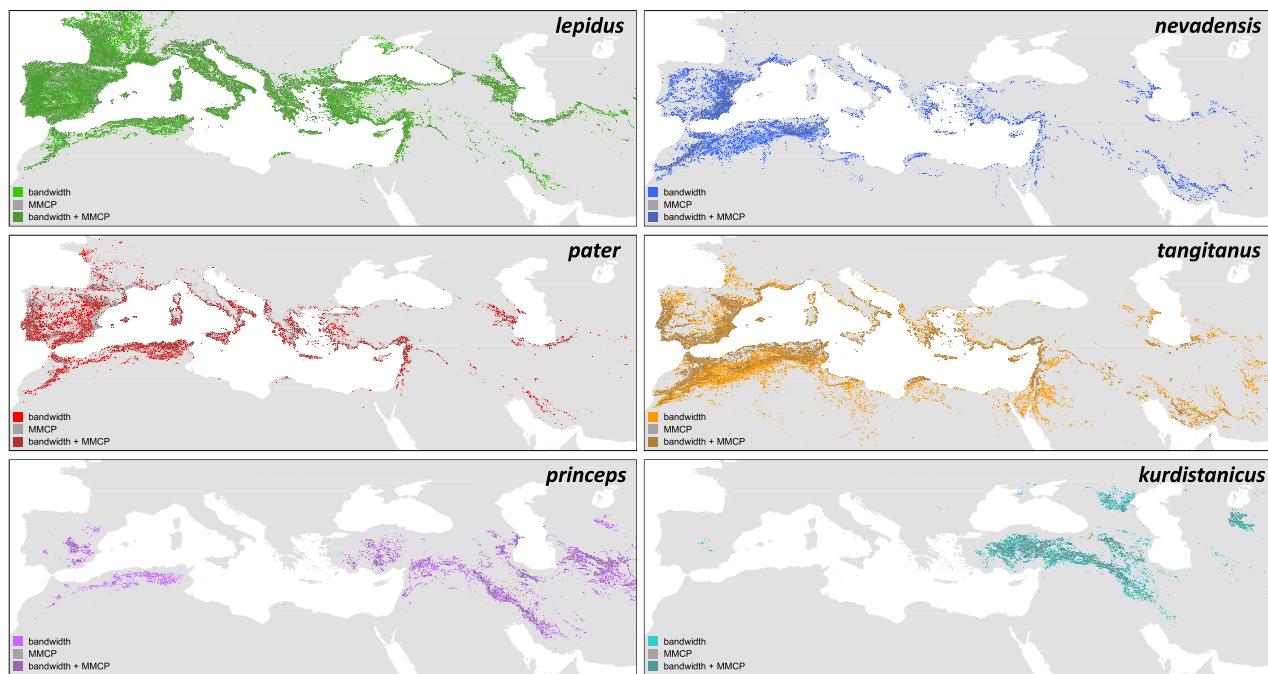


Figure 2 Species distribution models of *Timon* spp. derived from n -dimensional hypervolumes. Shown are projections of the hypervolumes for each species constructed using the bandwidth and the multivariate minimum convex polygon (MMCP) method.

Table 1 Pairwise niche overlap values in terms of D , equivalency p-values via randomization test and niche similarity P -values (Broennimann *et al.*, 2012), species hypervolumes, total hypervolume, intersection and Soerensen overlap (Blonder *et al.*, 2014) of *Timon* species (Tle = *T. lepidus*, Tne = *T. nevadensis*, Tpa = *T. pater*, Tpr = *T. princeps*, Tta = *T. tangitanus*, Tku = *T. kurdistanicus*). Statistically significant values are in bold. Soerensen indices range from 0 (no overlap) to 1 (niches are identical): $S = 2 * \text{intersection} / (\text{unique Volume } x + \text{unique Volume } y)$. Values given for the high dimension approach before and after the slash were obtained using bandwidth and multivariate minimum convex polygons respectively.

Low dimension (Broennimann <i>et al.</i> , 2012)					High dimension (Blonder <i>et al.</i> , 2014)				
Comparison	Niche overlap	Equivalency*	Similarity $x \rightarrow y$	Similarity $y \rightarrow x$	Volume x	Volume y	Volume union	Intersection	Soerensen overlap
Tle-Tne	0.2	$P < 0.0198$	0.16	0.69	303.41/134.75	11.52/22.98	309.06/168.89	5.86/11.17	0.04/0.14
Tle-Tpa	0.34	$P < 0.0198$	0.02	0.28	303.41/133.53	44.71/14.71	304.04/160.66	44.08/12.42	0.25/0.15
Tle-Tpr	0.14	$P < 0.0198$	0.02	0.89	303.41/144.26	1.72/10.35	305.13/156.09	0.00/1.48	0.00/0.02
Tle-Tta	0.24	$P < 0.0198$	0.02	0.63	303.41/130.10	37.81/32.33	314.45/178.22	26.77/15.78	0.16/0.18
Tne-Tpa	0.2	$P < 0.0198$	0.65	0.71	11.52/28.99	44.71/21.83	52.78/56.16	3.45/5.34	0.12/0.18
Tne-Tpr	0.22	$P < 0.0198$	0.02	0.24	11.52/32.59	1.72/10.27	13.24/44.41	0.00/1.55	0.00/0.07
Tne-Tta	0.31	$P < 0.0198$	0.02	0.14	11.52/20.10	37.81/34.15	41.41/68.33	7.92/14.08	0.33/0.35
Tpa-Tpr	0.01	$P < 0.0198$	0.4	0.02	44.71/26.14	1.72/10.81	46.43/38.00	0.00/1.05	0.00/0.06
Tpa-Tta	0.52	$P < 0.0198$	0.02	0.02	44.71/19.54	37.81/40.64	63.35/67.81	19.18/7.62	0.46/0.20
Tpr-Tta	0.04	$P < 0.0198$	0.53	0.32	1.72/10.27	37.81/46.72	39.53/58.55	0.00/1.57	0.00/0.05
Tku-Tle	0.19	$P < 0.0198$	0.5	0.02	9.57/23.33	303.41/145.86	312.98/169.35	0.00/0.16	0.00/0.00
Tku-Tne	0.16	$P < 0.0198$	0.06	0.02	9.57/23.44	11.52/34.14	21.09/57.57	0.00/0.00	0.00/0.00
Tku-Tpa	0.26	$P < 0.0198$	0.02	0.02	9.57/23.48	44.71/27.18	54.29/50.66	0.00/0.00	0.00/0.00
Tku-Tpr	0.28	$P < 0.0198$	0.02	0.2	9.57/22.30	1.72/10.60	11.29/34.09	0.00/1.19	0.00/0.07
Tku-Tta	0.22	$P < 0.0198$	0.06	0.46	9.57/23.47	37.81/48.33	47.39/71.80	0.00/0.00	0.00/0.00

indicating that niches are not identical between species. The randomization test of niche similarity revealed significant differences in 13 of 30 cases. Therefore, the observed niche overlap cannot be explained as simply deriving from availability of environmental space, but rather represents a function of habitat selection.

The n -dimensional hypervolume approach shows different results than the ‘PCA-env’ approach. While the ‘PCA-env’ approach considers only overlaps in two dimensions in all pairwise comparisons, the n -dimensional hypervolume (using either bandwidth or MMCP) detected four pairwise comparisons without any overlap (0.00), i.e. those between

T. kurdistanicus and each of the four western species (*T. tangitanus*, *T. lepidus*, *T. nevadensis* and *T. pater*). Using the *n*-dimensional approach, the highest intersection volume was measured between *T. lepidus* and *T. tangitanus*, but the two dimensions method revealed the highest overlap between the two African species. Among sister species, the lowest intersection volume belongs to the two eastern species (*T. kurdistanicus* and *T. princeps*, 1.19), whereas the two European species *T. lepidus* and *T. nevadensis* share the lowest overlap in the two-dimension method (Table 1).

Considerable divergences regarding the environmental tolerances were observed between the eastern and western clades in all principal components of the phyloclimatic reconstructions, except for PC1 (Fig. 4). In PC1, environmental tolerances evolve convergently between clades, but divergently within clades. PC2 suggests that the niches of all sister species have differentiated to some extent, while niches of European and African subclades are convergent between them but divergent from those of the eastern clade. In comparison to PC1, climatic tolerances of the species are gener-

ally narrower in PC2. Phyloclimatic reconstructions across PC3 show a pattern intermediate of PC1 and PC2, with niche divergence among clades (in different direction compared to PC2) and moderate divergence within clades. The inferred history from PC4 is similar to that observed in PC2, but with less divergence between the two eastern species and broader tolerances in two western species, *T. pater* and *T. lepidus*. The two European sister species do not show any obvious niche differentiation, as the environmental tolerance of *T. nevadensis* captured in PC4 is entirely nested within the broad ecological niche of *T. lepidus*.

DISCUSSION

Arnold *et al.* (2007) suggested that the Palaearctic true lizards (tribe Lacertini) radiated into the currently recognized genera during the mid-Miocene (12–16 Ma) when they experienced rapid speciation. Based on substantial differences in mitochondrial DNA, Arnold *et al.* (2007) proposed that *Timon* and *Lacerta* underwent an early divergence and geo-

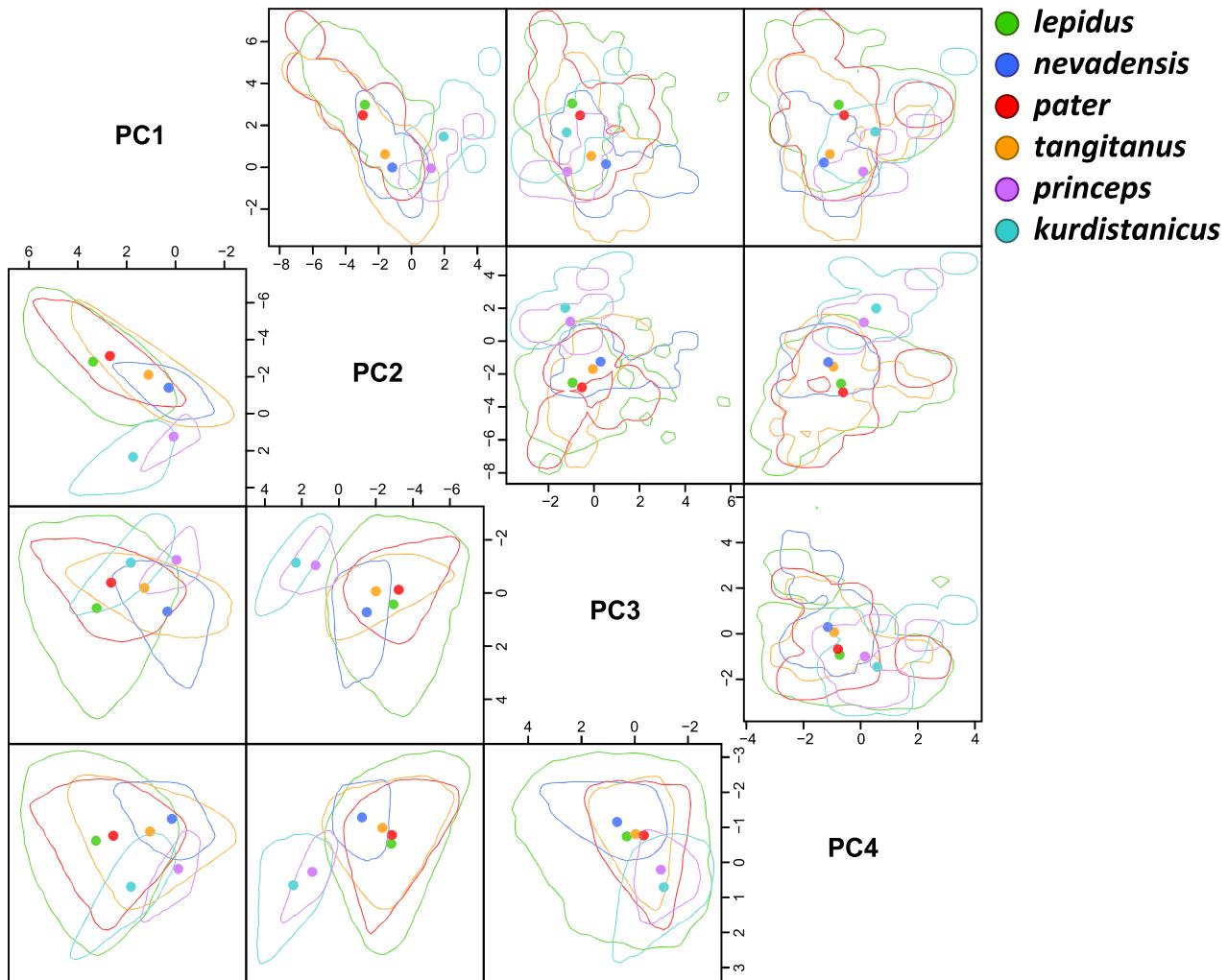


Figure 3 Four-dimensional hypervolumes of the environmental niches of *Timon* spp. Results for the bandwidth approach are above and for the multivariate minimum convex polygon approach are below the diagonal.

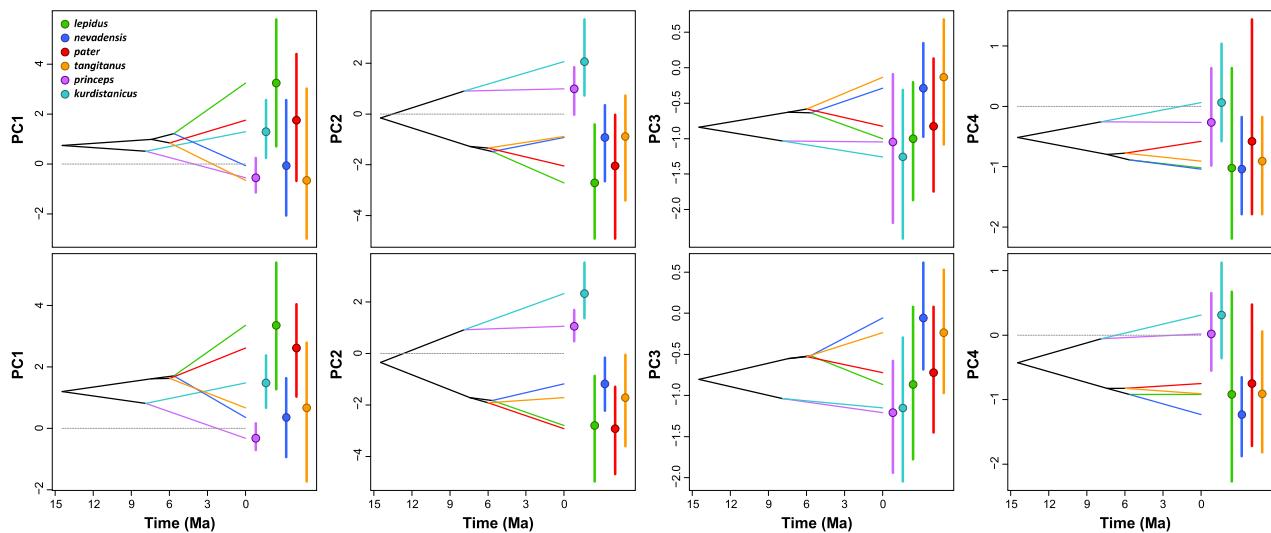


Figure 4 Inferred history of the evolution of environmental tolerances in the genus *Timon* based on the maximum a posteriori topology produced by the *BEAST analysis (see Fig. 1). Internal nodes represent the mean of environmental tolerances as inferred for the most recent common ancestor of the extant taxa defined by that node. The 80% central density of environmental tolerance for each extant taxon is indicated by vertical bars and points mark the respective mean values.

graphical spread. However, our results suggest an older age for this separation, which may be explained by the more comprehensive set of markers used here and the use of fossil-based calibration points (see also Hipsley *et al.*, 2009). The current allopatric and disjunct distribution of the genera within the Lacertini suggests multiple vicariant events. These may have caused their initial diversification, followed by a relatively fast geographical spread combined with separation into a number of taxonomic units that subsequently have remained largely or entirely allopatric at the generic level (Carranza *et al.*, 2004; Arnold *et al.*, 2007; Pavlicev & Mayer, 2009). Most likely, the ancestor of *Timon* diverged into the eastern and western groups following this pattern. This biogeographical patterns are well known for other closely related amphibians and reptiles: i.e. spadefoot toads of the genus *Pelobates* (*P. syriacus* vs. *P. cultripes* and *P. varaldii*) and parsley frogs of the genus *Pelodytes* (*P. caucasicus* vs. *P. punctatus* and *P. ibericus*) are found in both sides of the Mediterranean region (Tarkhnishvili & Gokhelasvili, 1999; Crottini *et al.*, 2010; Van de Vliet *et al.*, 2012). The species of worm lizards belonging to the genus *Blanus* (*B. strauchi* vs. *B. cinereus*, *B. mettetali*, *B. mariae* and *B. tingitanus*) and cylindrical skinks of the genus *Chalcides* (*C. guentheri* vs. *C. chalcides*) are other examples (Vasconcelos *et al.*, 2006; Albert *et al.*, 2007; Sindaco & Jeremcenko, 2008; Sindaco *et al.*, 2014; Sampaio *et al.*, 2015).

Phylogenetic results support our hypothesis, but the underlying ecological factors that promoted divergence and shaped the extant distribution of the species remain unknown. Our results indicate high complexity (i.e. observed patterns vary between niche dimensions and between clades) regarding the ecological niche evolution of *Timon* species between Mediterranean and non-Mediterranean climatic

regions. Currently, there is no evidence of sympatry between members of the genus. Nevertheless, the SDMs suggest overlap of the potential distributions of species within both eastern and western clades. The overall environmental niche overlap in the pairwise comparisons is generally low. Hence, the niche equivalency hypotheses were rejected in all cases, indicating species niches to possess significant differences. However, the similarity tests were not rejected in all cases, which indicate that the available environmental space within the general area of occurrence may contribute to the observed niche overlap patterns.

Analysing the niche occupancies in a phylogenetic context reveals different trends of niche evolution for the eastern and western clades of *Timon*. In particular, the species of the western clade show divergent evolution within a broad spectrum of ecological tolerance. The relatively deep niche divergence (except PC4) between the two European species might be responsible for the narrowness of their secondary contact zone (Miraldo *et al.*, 2011, 2012), suggesting that biotic interactions have shaped the distribution ranges between these species, which are currently not separated by any geographical barriers. Initial diversification between *T. lepidus* and *T. nevadensis* may well have been caused by allopatry (Miraldo *et al.*, 2011, 2012), although the isolation of the Baetic range (currently inhabited by *T. nevadensis*) from the Iberian mainland (*T. lepidus*) during the late Miocene (Paulo *et al.*, 2008) pre-dates the estimated divergence times. However, divergence roughly coincides with the Messinian salinity crisis (MSC; Krijgsman *et al.*, 1999), which caused a strong aridification of the southern Iberian Peninsula. This climatic change has been claimed to be responsible for vicariance in other lizards (e.g. *Podarcis*; Pinho *et al.*, 2006; Kaliontzopoulou *et al.*, 2011). Selective pressure across the landscape

likely caused locally divergent evolutionary responses of populations, resulting in divergent environmental tolerances (Ahmadzadeh *et al.*, 2013) and subsequently speciation. The high degree of niche differentiation between *T. lepidus* and *T. nevadensis*, especially in PC1 (Fig. 4), which summarizes the annual average amount of water content of vegetation (i.e. the annual mean of Middle Infra-Red), highlights the importance of vegetation cover and humidity during their niche evolution. The above-mentioned aridification of southern Iberia during the time these species evolved could be responsible for a rapid niche shift. Thus, these sister species show rather distinct habitat preferences, with *T. nevadensis* occupying more arid habitats.

A similar pattern of speciation due to allopatry and later niche divergence after secondary contact was established is inferred to the two African species, although these exhibit a larger overlap in environmental tolerances and lower degree of niche divergence (Fig. 4). As observed for the European species, geological changes in the western Mediterranean region during the late Miocene played a major role in diversification of the African clade, in particular the formation of the Tellian and Atlas systems (Paulo *et al.*, 2008). Considering the phylogeography of the group (high genetic divergence between populations of *T. tangitanus* from different mountain ranges; Perera & Harris, 2010) and the higher degree of niche overlap in these two African species, allopatry may have been the main factor for speciation in this clade. Divergence between the African and European clades predates the MSC, confirming the pre-MSC overseas dispersal hypothesis (Paulo *et al.*, 2008), if colonization of Africa and the Baetic-Rifian Archipelago from Europe is assumed. The greater dispersal ability of these large-bodied lizards enables them to occupy broader niches (Perera & Harris, 2010). As a result, one species is found across a Mediterranean-arid African climate range (*T. pater*) while the other species inhabits the Rif Atlas Mountains (*T. tangitanus*). Both regions have been affected by the drier climate of continental Africa and this pattern indicates adaptation to a wide range of environmental factors.

Accordingly, the predicted potential distributions, especially those of *T. lepidus* and *T. pater*, exceed their realized distribution under current climate conditions and encompass regions that were historically occupied. Several fossil records (Kotsakis, 1977; Böhme & Zammit-Maempel, 1982; Salotti *et al.*, 1996; Delfino & Bailon, 2000) that can be assigned to *Timon* (Mateo, 2011), but are far outside the current range (Fig. 1) provide a hint on a wider distribution of the genus during the Pleistocene. Furthermore, there are relict populations of *T. pater* and *T. tangitanus*, which have gone extinct only recently (Joger, 1981; Mateo *et al.*, 2004), and support the hypothesis of a historically wider distribution.

Regarding the time of separation, the eastern species display an earlier split than the western taxa. As discussed in Ahmadzadeh *et al.* (2012), divergence between the two eastern species was probably triggered by the uplifting of the Zagros mountains 5–10 Ma (see also Abdakhmatov *et al.*,

1996; Macey *et al.*, 1998). Our results suggest that, compared to the western species, the niches of *T. princeps* and *T. kurdistanicus* have evolved very slowly. The generally low niche divergence among members of the eastern group and the remarkable climatic tolerance within the western group also points to an important role of seasonality of temperature in niche evolution, in a mediterranean and Atlantic climate context. Dry summers and cold winters characterize the environmental niche of the two eastern species. The evidence for either niche conservatism, or low divergence, in this group suggests that speciation took place in allopatry and ecological differences accumulated only after the speciation event (McCormack *et al.*, 2010).

In conclusion, the ocellated lizards provide another example of vertebrate radiation where niche shift (with or without vicariance) and niche conservatism alternate throughout their historical biogeography.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Taxa used for phylogenetic analyses and properties of the molecular data set.

Appendix S2 Species occurrence records and derived variables with summary of the principal component analysis used for calculating the SDMs of *Timon* species.

Appendix S3 Pairwise comparisons of niches in climatic space (PCA-env) of *Timon* species.

BIOSKETCH

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