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Biogeography and contemporary climatic differentiation among Moroccan *Salamandra algira*

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The opening of the Gibraltar land bridge occurred at the end of the Messinian Salinity Crisis approximately 5.3 Mya, and was one of the main causes of vicariance between European and north-west African amphibians, resulting in the origin of several new species. However, little is currently known about the causes for post-Messinian amphibian differentiation in the Maghreb, although it is acknowledged that the Pleistocene glaciations probably had considerable influence on several species. The current study uses both species distribution modelling (MAXENT) and information from a total of 694 bp of mitochondrial data (351 from cytochrome *b* and 342 from *12S* rRNA) from 36 representatives of all three recognized subspecies of Moroccan *Salamandra* to infer the phylogeny and biogeography of *Salamandra algira tingitana*, which is characterized by both viviparous and ovoviviparous populations. According to the results, the split between *S. a. tingitana* and *S. a. algira* from the Rif and Middle Atlas mountains took place approximately 1.6 Mya, and could have been caused by a shift towards a colder and drier climate that occurred during the upper Pliocene, which may have resulted in the isolation of *Salamandra* at increasingly higher altitudes, or in other climatically favourable areas. Several lineages within *S. a. tingitana* originated during the Pleistocene climatic oscillations, one of which gave rise to the viviparous populations north of the Oued Martil. It is suggested that the origin of viviparity in *S. a. tingitana* occurred during the last 600 000 years. In order to further understand the origin of the unique viviparous population of *S. algira* from North Africa, predictive distribution models of the viviparous and ovoviviparous populations of *S. a. tingitana* were created using MAXENT to assess environmental differences. Niche divergence was subsequently determined using Schoener's *D* and Warren *et al.*'s *I* niche similarity metrics. Predictive modelling and niche divergence analyses revealed significant environmental differences between the two reproductive types, which could have influenced the transition from ovoviviparity to viviparity. © 2010 The Linnean Society of London, Biological Journal of the Linnean Society, 2010, **101**, 626–641.

ADDITIONAL KEYWORDS: MAXENT – mitochondrial DNA – Morocco – phylogeography – species distribution modelling – viviparity.

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INTRODUCTION

The Palaearctic amphibian fauna of the Maghreb region (north-west Africa) has long been regarded as relatively poor and closely related to that of Europe. An increasing number of genetic studies have revealed high levels of endemism, however, mainly caused by the long-separated evolutionary history of European and North African Palaearctic amphibians (reviewed in Pleguezuelos, Fahd & Carranza, 2008). The main isolative event leading to this current situation seems to be the opening of the Gibraltar land bridge and the subsequent filling of the Mediterranean Basin at the end of the Messinian Salinity Crisis (MSC). For amphibians, this event was the principal cause of vicariance between populations of the genera *Pleurodeles*, *Salamandra*, *Pelobates*, *Alytes*, and *Discoglossus* on each side of the Strait of Gibraltar (Steinfartz, Veith & Tautz, 2000; García-París, Buchholz & Parra-Olea, 2003a; Carranza & Wade, 2004; Martínez-Solano *et al*., 2004; Zangari, Cimmaruta & Nascetti, 2006). Long-standing marine transgressions after the breach of the Gibraltar land bridge caused several known isolative events, which triggered speciation in the western Mediterranean (e.g. Capula *et al*., 1985; Arntzen & García-París, 1995; Mattoccia, Romano & Sbordoni, 2005), with the Pliocene differentiation of *Pleurodeles poireti* Gervais, 1835 on an Algerian 'fossil island' being one of the few examples demonstrating the effect of these events on Maghrebian amphibians (Carranza & Arnold, 2004; Carranza & Wade, 2004). The Pleistocene glaciations also seem to have influenced the differentiation of some Maghrebian amphibian populations, although only a few cases are currently known (Carranza & Arnold, 2004; Batista *et al*., 2006; Zangari *et al*., 2006).

In recent years our increased understanding of the North African fire salamander *Salamandra algira* Bedriaga, 1883 has revealed considerable post-Messinian differentiation and speciation, which has resulted in the identification of several subspecies (Donaire-Barroso & Bogaerts, 2003; Escoriza & Comas, 2007). One of these subspecies, *Salamandra algira tingitana* Donaire-Barroso & Bogaerts, 2003, is characterized by a total absence of red coloration, whereas all other Moroccan *Salamandra* are characterized by ontogenetic development of both yellow and red coloration. Males of *S. a. tingitana* are also unique in having a very high number of glands on the skin during the mating period (Donaire-Barroso & Bogaerts, 2003). These phenotypical characteristics are paralleled by considerable genetic divergence, as demonstrated by Escoriza *et al*. (2006), which has led some authors to consider *S. a. tingitana* as a full species (Dubois & Raffaëlli, 2009).

Salamandra algira tingitana is both ecologically and morphologically very variable. It presents two main forms: one that lives north of the Oued Martil (see Fig. 1), and is characterized by being viviparous and by an ontogenetic transition of the yellow colour pattern towards hypoluteism or melanism (Donaire-Barroso & Bogaerts, 2001); and another form living on both sides of the Oued Hajera, in the area bordered by the Oued Martil, near Tetouan, in the north and the Oued Laou, near Chefchaouen, in the south (see Fig. 1), which is ovoviviparous and does not present a tendency towards hypoluteism (Donaire-Barroso & Bogaerts, 2003). As in other examples, the viviparous reproductive behaviour found in the form of *S. a. tingitana* north of the Oued Martil could be caused by environmental pressures (e.g. Gasser & Joly, 1972; Velo-Antón *et al*., 2007), as hypothesized by Donaire-Barroso & Bogaerts (2003), although the exact origin is unknown.

Despite several attempts, the evolutionary history and relationships of *S. algira* in the extreme north-west of the Maghreb are still not fully understood. For instance, the ovoviviparous populations of *S. a. tingitana* and the southernmost populations of *S. a. algira* from the Middle Atlas mountains (Fig. 1) have never been included in any phylogenetic analysis, and so their relationships with the viviparous populations north of the Oued Martil and with all the rest of *S. algira* from Morocco are unknown (Steinfartz *et al*., 2000; Donaire-Barroso & Bogaerts, 2003; Escoriza *et al*., 2006).

In this study we aim: (1) to investigate the systematics and biogeography of the north-western Moroccan *Salamandra*; and (2) to assess whether there are environmental differences between the distributions of the two reproductive types found in *S. a. tingitana*.

MATERIAL AND METHODS

GENETICS

Specimens analysed

A total of 49 specimens were incorporated into the phylogenetic analyses, including 36 representatives of all three known subspecies of Moroccan *Salamandra* (five *S. a. tingitana* from the viviparous populations situated to the north of Oued Martil, eight *S. a. tingitana* from the ovoviviparous populations bordered by the Oued Martil to the north and the Oued Laou to the south, 13 *S. a. algira* from the Rif mountains, seven *S. a. algira* from the Atlas mountains, and three *Salamandra algira spelaea* Escoriza & Comas, 2007 from the Beni Snassen massif. Additionally, six European *Salamandra* were also included. *Ambystoma tigrinum tigrinum* was used to root the tree and six representatives of the genus *Pleurodeles*

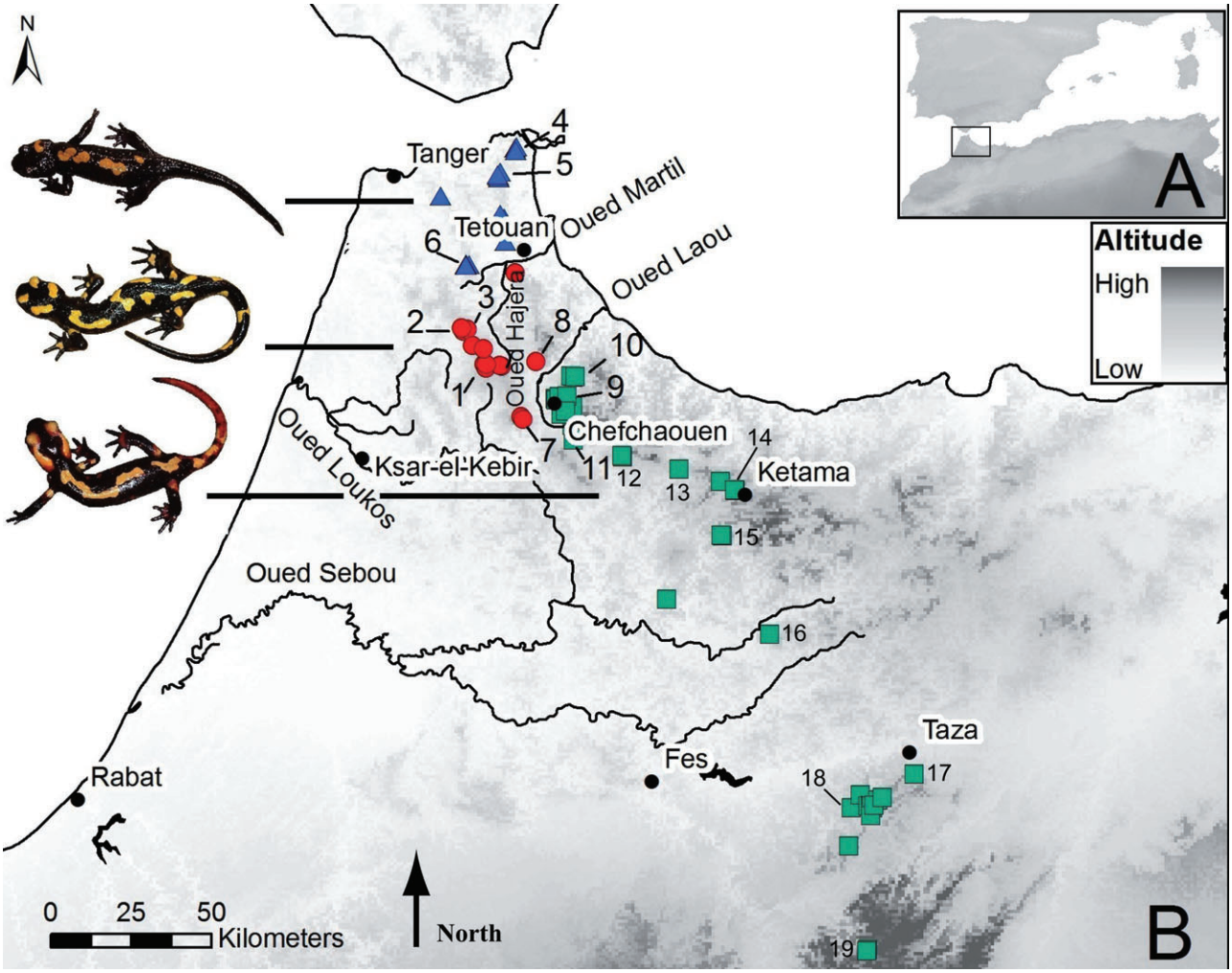

Figure 1. A, location of the study area in the western Mediterranean. B, elevation map showing the distribution of *Salamandra* in north-western Morocco, major Oueds, and main cities. Blue triangles, viviparous populations of *Salamandra algira tingitana*; red circles, ovoviviparous populations of *S. a. tingitana*; green squares, ovoviviparous populations of *Salamandra algira algira* (not used for modelling). The numbers indicate the populations of north-western Moroccan *Salamandra* included in the phylogenetic analyses (see Table 1).

were included for calibration purposes. Details on the locality and GenBank accession numbers of all specimens sequenced are given in Table 1. Localities of the north-western Moroccan samples included in the phylogenetic analyses are shown in Figure 1.

Extraction of DNA and PCR amplification

The DNeasy tissue kit was used to extract genomic DNA from the samples, following the manufacturer's instructions (Qiagen). Primers used in both amplification and sequencing were specifically designed for this project, and had the following sequence: SalaCytbF 5′-CTAATGACCCACATCCTTCGAAAAAC AC-3′, SalaCytbR 5′-TGGGAGTACGTATCCTACAAA GGCTG-3′ for the mitochondrial cytochrome *b* (cyt*b*) gene; and the Sala12F 5′-GATACCCCACTATGCCTA GCCATAAAC-3′, Sala12R 5′-GGGCGGTGTGTGCG CGCTTTATTGC-3′ for the mitochondrial *12S* rRNA gene. The two gene fragments were amplified using the same PCR conditions: an initial step of 5 min at 94 °C, followed by 35 cycles of 30 min at 94 °C; 45 min at 48 °C, and 60 min at 72 °C; with a final step of 5 min at 72 °C. Successful polymerase chain reaction (PCR) products were sequenced with an ABI 377 automated sequencer following the manufacturer's protocols.

Phylogenetic analyses

DNA sequences were aligned using CLUSTALX (Thompson *et al*., 1997) with default parameters (gap opening = 10; gap extension = 0.2). Only a few gaps had to be postulated to unambiguously align all the

Table 1. Details of specimens and sequences used in the present study

Taxa	Locality	Accession numbers Cyt *b*/12S rRNA	Codes
Salamandra algira spelaea 1	Beni Snassen (Morocco)	DQ901444/HQ190867	E220364
Salamandra algira spelaea 2	Beni Snassen (Morocco)	DQ901443/HQ190866	E220363
Salamandra algira spelaea 3	Beni Snassen (Morocco)	DQ901442/————	E220362
Salamandra algira tingitana 4	Jebel Suna (Morocco) [7]	HQ190882/HQ190852	E13053.16
Salamandra algira tingitana 5	Beni Maharone (Morocco) [8]	HQ190881/HQ190851	E13053.13
Salamandra algira tingitana 6	Ain Lahsen (Morocco) [6]	HQ190886/HQ190855	E3009.9
Salamandra algira tingitana 7	Jebel El Fahies (Morocco) [4]	HQ190883/————	E309.13
Salamandra algira tingitana 8	Jebel El Fahies (Morocco) [4]	AY247732/DQ221227	E1712.7
Salamandra algira tingitana 9	Tagramt (Morocco) [5]	HQ190885/HQ190854	E309.12
Salamandra algira tingitana 10	Tagramt (Morocco) [5]	AY247733/DQ221228	E309.10
Salamandra algira tingitana 11	Tazia crossing (Morocco) [2]	HQ190878/HQ190848	E1305.15
Salamandra algira tingitana 12	S. Moulay Abdessalam (Morocco) [1]	HQ190877/HQ190847	E2909.21
Salamandra algira tingitana 13	S. Moulay Abdessalam (Morocco) [1]	HQ190876/HQ190846	E2909.20
Salamandra algira tingitana 14	Beni Arous (Morocco) [3]	HQ190879/HQ190849	E17121.8
Salamandra algira tingitana 15	Beni Arous (Morocco) [3]	HQ190879/HQ190849	E3009.11
Salamandra algira tingitana 16	Beni Arous (Morocco) [3]	HQ190880/HQ190850	E30092.8
Salamandra algira tingitana 17	Taza, Ras el ma (Morocco) [17]	HQ190891/HQ190860	E17121.13
Salamandra algira tingitana 18	Taza, Ras el ma (Morocco) [17]	HQ190890/HQ190859	E17121.11
Salamandra algira tingitana 19	Taza, Ras el ma (Morocco) [17]	HQ190896/HQ190865	E15124.8
Salamandra algira tingitana 20	East of Bab Ahzar (Morocco) [18]	HQ190895/HQ190864	E15124.5
Salamandra algira tingitana 21	Taffert (Morocco) [19]	HQ190894/HQ190863	E15124.1
Salamandra algira tingitana 22	Taffert (Morocco) [19]	HQ190893/HQ190862	E13124.7
Salamandra algira tingitana 23	Taffert (Morocco) [19]	HQ190892/HQ190861	E13124.6
Salamandra algira algira 24	Mokrisset (Morocco) [11]	HQ190887/HQ190856	E1113.5
Salamandra algira algira 25	Mokrisset (Morocco) [11]	HQ190889/HQ190858	E1113.4
Salamandra algira algira 26	Mokrisset (Morocco) [11]	HQ190888/HQ190857	E13053.14
Salamandra algira algira 27	Askur, near Talembot (Morocco) [10]	AY247734/DQ221229	E1712.9
Salamandra algira algira 28	Cudia-Sbaa (Morocco) [12]	HQ190899/HQ190870	E15124.3
Salamandra algira algira 29	Chefchaouen (Morocco) [9]	AY247735/DQ221230	E1712.14
Salamandra algira algira 30	Jebel Aaoul (Morocco) [15]	HQ190901/HQ190872	E11113.6
Salamandra algira algira 31	Jebel Aaoul (Morocco) [15]	HQ190902/HQ190873	E11113.7
Salamandra algira algira 32	Ketama (Morocco) [14]	HQ190898/HQ190869	E11113.3
Salamandra algira algira 33	Ketama (Morocco) [14]	HQ190903/HQ190874	E13124.4
Salamandra algira algira 34	Ketama (Morocco) [14]	HQ190904/HQ190875	E13124.5
Salamandra algira algira 35	Bouadelle (Morocco) [16]	HQ190900/————	E15124.6
Salamandra algira algira 36	8 Km E. of Bab Berret (Morocco) [13]	HQ190897/HQ190868	E11113.2
Salamandra salamandra longirostris	Los Barrios (Spain)	DQ221243/DQ221223	E1712.37
Salamandra salamandra bernardezi	Tendi Valley (Spain)	DQ221240/DQ221220	E1712.20
Salamandra salamandra gigliolii	Sierra de San Bruno (Italy)	DQ221241/DQ221221	E1712.34
Salamandra salamandra fastuosa	Bagneres de Luchon (France)	DQ221234/DQ221214	E1712.27
Salamandra salamandra salamandra	Ukraine	DQ221232/DQ221213	E7110.15
Salamandra salamandra terrestris	Montseny (Spain)	AY222503/AY222459	E1712.49
Pleurodeles nebulosus	Larba (Algeria)	AY222504/AY222460	Anc10
Pleurodeles nebulosus	Constantine (Algeria)	AY222462/AY222506	Anc12
Pleurodeles nebulosus	Tabarka (Tunisia)	AY222518/AY222474	E181210
Pleurodeles poireti	Annaba (Algeria)	AY222507/AY222463	Anc13
Pleurodeles waltl	Extremos (Portugal)	AY222515/AY222471	E10114
Pleurodeles waltl	Perelló (Spain)	AY222531/AY222487	E181224
Ambystoma t. tigrinum		NC_006887	MtGenome

Numbers between square brackets after locality names refer to the localities from north-western Moroccan *Salamandra* shown in Figure 1.

12S rRNA sequences; therefore, all the positions were included in the phylogenetic analyses.

Topological incongruence among partitions was tested using the incongruence length difference (ILD) test (Michkevich & Farris, 1981; Farris *et al*., 1994). In this, 10 000 heuristic searches were carried out after removing all invariable characters from the data set (Cunningham, 1997). To test for incongruence among data sets we also used a reciprocal 70% bootstrap proportion (Mason-Gamer & Kellogg, 1996) or a 95% Bayesian posterior probability threshold. Topological conflicts were considered significant if two different relationships for the same set of taxa were both supported with bootstrap values of $>70\%$, or posterior probability values of $>95\%$.

Two methods were employed for phylogenetic analyses of the two separate gene fragments, and for those in which they were combined. These were maximum-likelihood (ML) and Bayesian analyses. JMODELTEST (Posada, 2008) was used to select the most appropriate model of sequence evolution for the ML and Bayesian analyses under Akaike's information criterion. This was the GTR model taking into account the gamma distribution and the number of invariant sites for each independent partition (cyt*b* and *12S*) and for the combined data set (cyt*b* + 12S).

The ML analyses were performed with PHYML (Guindon & Gascuel, 2003), with model parameters fitted to the data by likelihood maximization. Bayesian analyses were performed on MRBAYES v3.1.2 (Huelsenbeck & Ronquist, 2001), and each partition had its own model of sequence evolution and model parameters (see above). Four incrementally heated Markov chains with the default heating values were used. All analyses started with randomly generated trees and ran for 2×10^6 generations in two independent runs, with samplings at intervals of 100 generations that produced 20 000 trees. After verifying that stationarity had been reached, both in terms of likelihood scores and parameter estimation, the first 5000 trees were discarded in both independent runs of the cyt*b* and *12S* rRNA, and the combined analyses, and a majority rule consensus tree was generated from the remaining 15 000 post-burn-in trees. The frequency of any particular clade among the individual trees contributing to the consensus tree represents the posterior probability of that clade (Huelsenbeck & Ronquist, 2001): only values equal or above 95% were considered to indicate sufficient support (Wilcox *et al*., 2002).

In order to estimate divergence times between lineages R8S v1.7.1 was used (Sanderson, 1997, 2002). This program implements several methods for estimating absolute rates of molecular evolution, ranging from standard ML analyses to more experimental semiparametric and nonparametric methods, which relax the stringency of the clock assumptions using smoothing methods. In order to account for the error involved in the calibration of the *Salamandra* phylogeny, a parametric bootstrap analysis was performed in which we simulated 1000 alignments from the ML tree and recalculated dates using R8S from the same ML topology with branch lengths optimized for each simulated alignment. This allowed us to evaluate the stochastic errors of date estimates associated with sampling a finite number of base pairs. Analyses were performed as described in Sanderson & Doyle (2001) and Lalueza-Fox *et al*. (2005).

A number of amphibian and other terrestrial vertebrate groups with long fossil histories in Europe have distinctive taxa in the south-west of the continent. Several of these possess sister groups in north-west Africa, and phylogenies indicate that they invaded the area from Europe. The amphibian sister pairs show similar divergences in mitochondrial DNA (mtDNA). This is true of members of the salamandrid genus *Pleurodeles* (Carranza & Arnold, 2004) and of three anurans, *Alytes* (Martínez-Solano *et al*., 2004), *Discoglossus* (Zangari *et al*., 2006), and *Pelobates* (García-París *et al*., 2003a). The repeated phylogenetic pattern and similarity in degree of divergence makes it likely that the separation of all sister pairs was caused by the same vicariance event. Entry into north-west Africa and the vicariance event itself probably resulted from the MSC, as some of these taxa, e.g. *Discoglossus* and *Alytes*, also colonized some Mediterranean islands at the same time (Martínez-Solano *et al*., 2004; Zangari *et al*., 2006). As a result, the date of the opening of the Strait of Gibraltar (5.3 Mya) was used to calibrate the clock, which most probably was the vicariant event that caused the separation between European and North African newts of the genus *Pleurodeles* (Carranza & Arnold, 2004; Carranza & Wade, 2004).

Species distribution modelling

Presences and environmental data

A total of 30 GPS-georeferenced localities of *S. a. tingitana* north of the Oued Laou collected between 1995 and 2009 were used for distribution modelling (see Fig. 1). All presence data were used for modelling, regardless of clustering in several areas, and were divided into two reproductive types: (1) the viviparous populations of *S. a. tingitana* situated to the north of Oued Martil ($n = 16$), and (2) the ovoviviparous *S. a. tingitana* populations bordered by the Oued Martil to the north and the Oued Laou to the south ($n = 14$) (Figure 1).

The study area comprised the north-western part of the Moroccan Rif region, also known as the Tingitana Peninsula (from 35°55′N, 4°25′W to 34°19′N, 6°30′W).

A total of 19 BioClim variables were downloaded from the WorldClim database v1.4 (http://www.worldclim.org) to form the climatic data set (Hijmans *et al*., 2005). Topogeographical variables (altitude, slope, and aspect) were obtained from the US Geological Survey (http://edc.usgs.gov). The variable aspect was reclassified using the reclassify function in ARCGIS into four classes (north, east, south, and west). Normalized difference vegetation index (NDVI) images covering Morocco were used to assess the influence of the temporal behaviour of vegetation greenness on the possible differentiation and output of the models, as NDVI can considerably improve distribution models (e.g. Peterson & Nakazawa, 2008). The images contain maximum-value composite (MDV) data for each 10-day period from the 1st of April 1998 to the 1st of May 2008. Cluster separability evaluation generating minimum and average divergence statistics revealed that the best number of classes that can be extracted through unsupervised classification of the NDVI stack of images is 75 (Khan *et al*., 2010). The superficial, categorical geology (28 classes within the study area) of North Africa was downloaded from http://www.northafrica.de/gis.htm. All variables were downloaded at a resolution of nearly 1×1 km. Stepwise linear regression of the continuous variables for each subgroup was performed with SPSS 16 until the first variable reached a variance inflation factor (VIF) of less than 10 in order to exclude correlating variables (Belsley, Kuh & Welsch, 1980).

Model building

Maximum entropy modelling (MAXENT) of the geographic distributions of species was calculated with MAXENT v3.3.1 (Phillips, Anderson & Shapire, 2006a). It has been shown to produce high-quality predictions that are often more successful when evaluated and compared with other predictive models (Elith *et al*., 2006; Hernandez *et al*., 2006; Jiménez-Valverde, Lobo & Hortal, 2008). Additionally, MAXENT has a successful prediction power, even when using low sample sizes (Pearson *et al*., 2007; Wisz *et al*., 2008). This algorithm uses environmental parameters in combination with geographical coordinates in order to predict the distribution of the species of interest. Maximum entropy is achieved by the constraint that the expected value for each variable under the estimated distribution has to match its empirical average: in other words, the mean value of a random set of coordinates within the distribution (Phillips *et al*., 2006a). The model output displays the relative occurrence probability of a species within the grid cells of the study area. MAXENT was used with default settings while partitioning the geographical records between training and test samples (75 and 25%, respectively), a technique that has been proven to achieve high predictive accuracy (Phillips & Dudík, 2008). Ensemble forecasting (Araújo & New, 2007) of one hundred models for each reproductive type with randomly selected test samples was used to produce predictive distribution maps, which were plotted in logistic format. The final models were reclassified in ARCGIS into binary presence–absence maps, based on the average 10-percentile threshold given by MAXENT. Jackknife testing was used to produce estimates of the average contribution and response of each variable to the model. Thus, each variable was excluded in turn and a model with the remaining variables created. Additionally, a model using each variable in isolation and a model using all variables were created.

Model validation and interpretation

All models were tested with receiver operating characteristics (ROC) curve plots, which plot the true-positive rate against the false-positive rate. The average area under the curve (AUC) of the ROC plot of ten models was taken as a measure of the overall fit of each model. Because MAXENT operates with only presence records, the AUC is calculated using pseudo-absences chosen at random from the study area (Phillips *et al*., 2006a, b). The AUC values range between 0.00 (highly unsuitable) and 1.00 (highly suitable), and display the probability that a randomly chosen presence site will be ranked above a randomly chosen absence site (Phillips *et al*., 2006a, b). Models with AUC values above 0.75 are considered to be useful (Elith, 2002).

Visual analyses of the response curves and graphs of major contributing variables (Austin, 1987) produced by jackknife analyses were performed in order to assess how the probability of presence of each reproductive type changes with the range of variation within the predictor variables. Response curves were analysed from models created using only the corresponding variable. Distinct differences between reproductive types were taken as an indication of environmental divergence.

Niche conservatism or niche divergence

Environmental niche model tools (ENMTools; Warren, Glor & Turelli, 2010) was used to test whether the habitat suitability scores generated by distribution models from the two reproductive types of *S. a. tingitana* exhibited statistically significant ecological differences (following the methods of Warren, Glor & Turelli, 2008). Two niche similarity metrics were first calculated using a test for niche overlap: Schoener's *D* and Warren *et al*.'s *I*. Niche similarity is quantified statistically from 0 (no overlap) to 1 (identical niche models), based on the predictive distribution of both reproductive types. A niche identity test with 100

replicates, which pools empirical occurrence points and randomizes their identities to produce two new samples with the same number of observations as the empirical data, was then implemented. The obtained overlap scores represent the expected degree of niche overlap (both *D* and *I*) when samples are drawn from the same distribution. These data were compared with the initial niche overlap test based on the actual occurrences to determine whether the two reproductive types are more different than would be expected by chance.

RESULTS

Phylogenetic analyses

The two gene partitions used (cyt*b* and *12S*) were shown to be congruent using the ILD test ($P = 0.70$), and independent analyses of these gene partitions confirmed that there were no topological conflicts (Mason-Gamer & Kellogg, 1996). Both mitochondrial fragments were therefore combined for further analyses. Of the 694 bp in the combined data set (351 from cyt*b* and 342 from *12S*), 206 were variable and 151 were parsimony-informative. If only the 36 *S. algira* from Morocco were considered, the number of variable sites decreased to 68 for the combined data set (48 for cyt*b* and 20 for *12S*), and the number of parsimony-informative decreased to 58 (43 for cyt*b* and 15 for *12S*). The results of the phylogenetic analyses are shown in Figure 2. Both ML and Bayesian analyses produced trees with identical topologies in which the European and Moroccan *Salamandra* form reciprocally monophyletic groups. Within Morocco, *S. a. spelaea* is basal. The ovoviviparous *S. a. tingitana* populations from the area between the Oued Martil and Oued Laou (see Fig. 1) form a monophyletic group with the viviparous *S. a. tingitana* populations situated to the north of Oued Martil. In fact, as shown in Figure 2, the *S. a. tingitana* specimens from the ovoviviparous populations of Beni Hassan and Jebel Suna are sister to the remaining ovoviviparous and viviparous populations further north, which suggests a single evolutionary origin for the viviparous *S. a. tingitana* populations from the ovoviviparous populations of the same subspecies. It is also shown that the populations of *S. a. algira* from the Rif and Middle Atlas mountains form an independent and well-supported lineage sister to *S. algira tingitana*. This lineage is further subdivided into two clades. One clade containing the populations from the Rif and another one the populations from the Middle Atlas (see Figs 1, 2). For the cyt*b* gene, the levels of genetic differentiation between the Moroccan populations of *Salamandra* range between 1.15 (genetic distance between ovoviviparous and viviparous populations of *S. al. tingitana*) and 7.56% (genetic distance between population of *S. a. algira* from the Rif mountains and *S. a. spelaea* from the Beni Snassen). Genetic distances for the cyt*b* and *12S* genes are presented in Appendix 1.

Distribution modelling

Ensemble forecasting produced highly predictive accuracy models according to the average testing AUC for the viviparous populations (0.997 ± 0.002) and the ovoviviparous populations (0.995 ± 0.005). The main predictor variables of the *S. a. tingitana*

Table 2. Average percentage contributions ± SDs of the predictor variables and validation for the two reproductive types of *Salamandra algira tingitana*

Predictor variables	*S. a. tingitana* viviparous	*S. a. tingitana* ovoviviparous
Normalized Difference Vegetation Index (NDVI)	**24.0121 ± 10.1386**	**45.9989 ± 8.0233**
Geology	**29.2414 ± 11.2085**	0.6468 ± 0.8238
Altitude	0.0254 ± 0.1063	0.0134 ± 0.0589
Aspect	0.0167 ± 0.0352	0.0240 ± 0.1951
Slope	0.0111 ± 0.0542	0.1079 ± 0.4662
Mean Diurnal Range: Mean of monthly (max temp – min temp)	**29.1420 ± 5.3570**	0.1016 ± 0.1626
Max Temperature of Warmest Month	2.887 ± 2.1620	2.4746 ± 1.1406
Mean Temperature of Wettest Quarter	0.0160 ± 0.0662	0.1808 ± 0.4378
Mean Temperature of Driest Quarter	0.4288 ± 0.5520	0.1883 ± 0.3349
Annual Precipitation	1.5738 ± 3.2624	8.9890 ± 5.4182
Precipitation of Driest Quarter	1.8683 ± 1.6111	0.2571 ± 0.3708
Precipitation of Coldest Quarter	10.5756 ± 3.4359	**37.5276 ± 13.9084**
Testing AUC	0.997 ± 0.002	0.995 ± 0.005

Bold numbers represent main predictor variables.

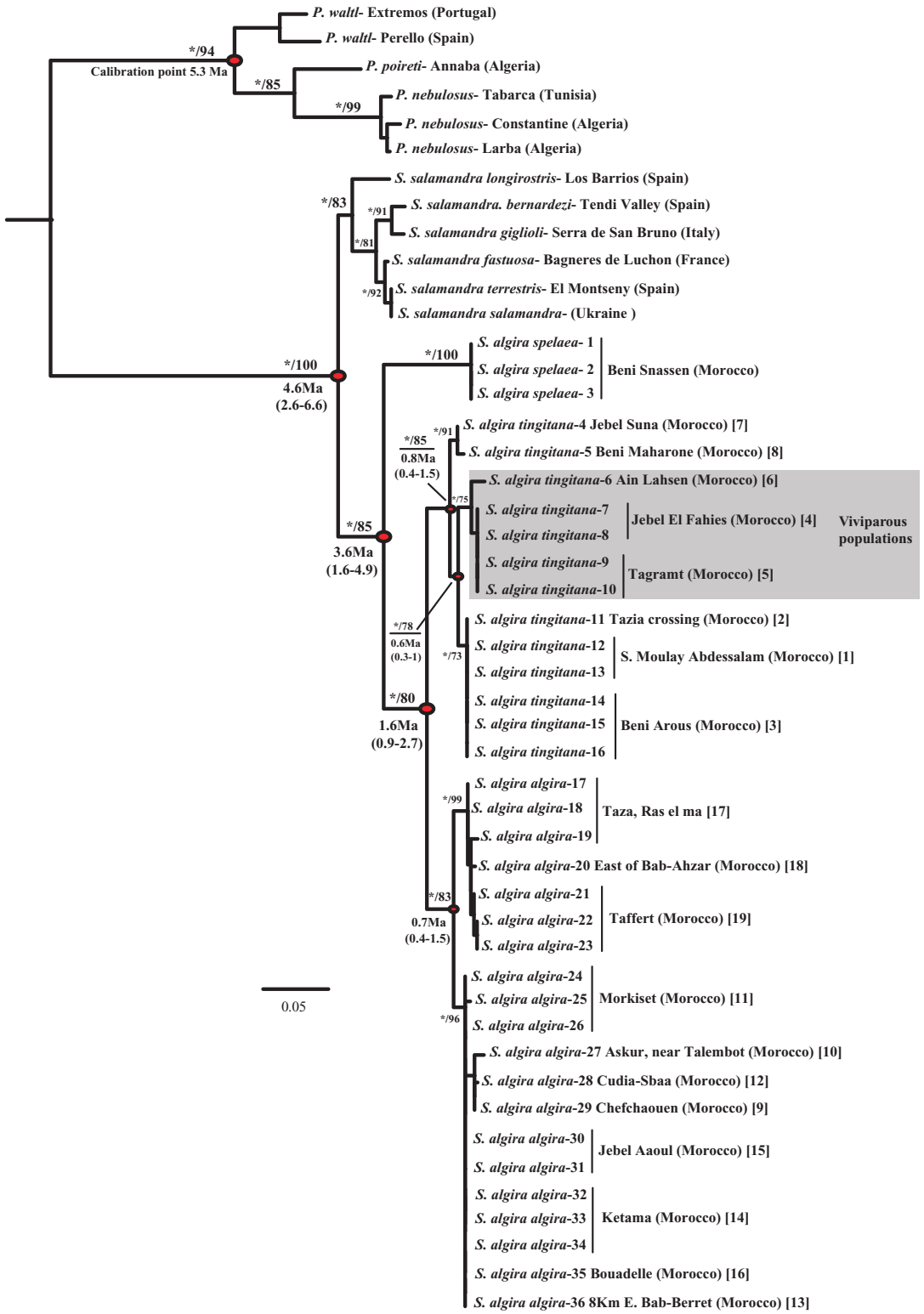

Figure 2. Phylogenetic relationships of north-west African *Salamandra* based on 351 bp of cytochrome *b* and 342 bp of *12S* rRNA mitochondrial genes. *Ambystoma tigrinum tigrinum* (not shown on the tree) was used to root the tree and the sharp-ribbed newts *Pleurodeles waltl*, *Pleurodeles poireti*, and *Pleurodeles nebulosus* were used to calibrate the tree (see Material and methods). Numbers between square brackets after locality names refer to the sampling localities shown in Figure 1.

viviparous populations are NDVI, geology, and mean temperature range [mean of monthly (max. temp – min. temp)] (see Table 2). In contrast, only two variables, namely NDVI and precipitation during the coldest quarter, are main predictors for the *S. a. tingitana* ovoviviparous populations (see Table 2). Visual analyses of the response curves derived from the continuous variables revealed that the distribution of the viviparous populations is related to a smaller and lower mean temperature range compared with that of the ovoviviparous populations (Appendix 2). Additionally, the probability of the presence of the viviparous populations is characterized by a lower level of precipitation in the coldest quarter compared with that of the ovoviviparous populations. As for the categorical variables, visual analysis of the superficial geology categories for both groups revealed a high probability of presence on Palaeozoic, Mesozoic, Tertiary, and Cretaceous soils for the viviparous populations, and Mesozoic, Tertiary, and Cretaceous soils for the ovoviviparous populations (Fig. 3a, b). Three of the 75 vegetation classes (classes 66, 72, and 75) from NDVI are related to the suitable distribution of both *S. a. tingitana* reproductive types (Fig. 3b).

The predicted distribution of both reproductive types largely corresponds to the known distribution determined from the spread of presence localities (Fig. 4). The suitable habitat for the viviparous populations, however, seems to continue southwards along the eastern slopes of the central mountains. The suitable habitat of the ovoviviparous populations is mainly located on the central and western slopes of the same mountains. Areas of sympatry were not identified by the models (see Fig. 4). There is considerable overprediction of the ovoviviparous populations east of the Oued Laou, within the distribution range of *S. a. algira* near Chefchaouen and the central Rif (Fig. 1), thus indicating environmental similarities. The mountainous areas north-east of Ksar-el-Kebir surrounded by the Oued Loukos drainage, where no presence localities are known, are predicted to be highly suitable for the ovoviviparous *S. a. tingitana* (Fig. 4).

Niche conservatism or niche divergence

The initial niche overlap test based on the actual distribution data of both reproductive types scored 0.43 for Schoener's *D* and 0.56 for Warren *et al.*'s *I*. Subsequently, the identity test based on 100 pseudoreplicates produced niche overlap scores (Fig. 5). The niche overlap scores of the initial overlap test display a higher divergence than those of the identity test based on 100 replicates, thus showing that the two reproductive types differ more than would be expected by chance.

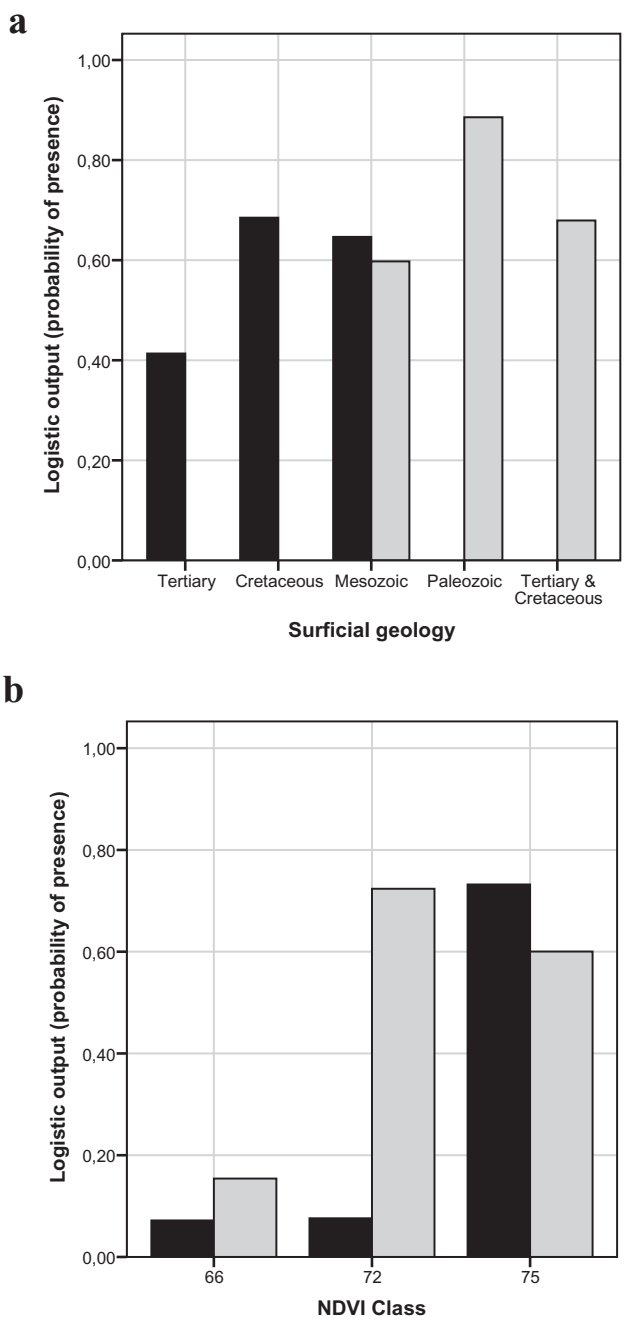

Figure 3. Response graphs for the major categorical predictor variables of the ovoviviparous (black) and viviparous (grey) populations of *Salamandra algira tingitana*.

DISCUSSION

The prediction of ecologically specialized species tends to be more accurate compared with the prediction of generalist species occupying a diversity of habitat types (e.g. Jiménez-Valverde *et al.*, 2008), thereby explaining the exceptionally high predictive accuracy (AUC value) of each model. Despite the

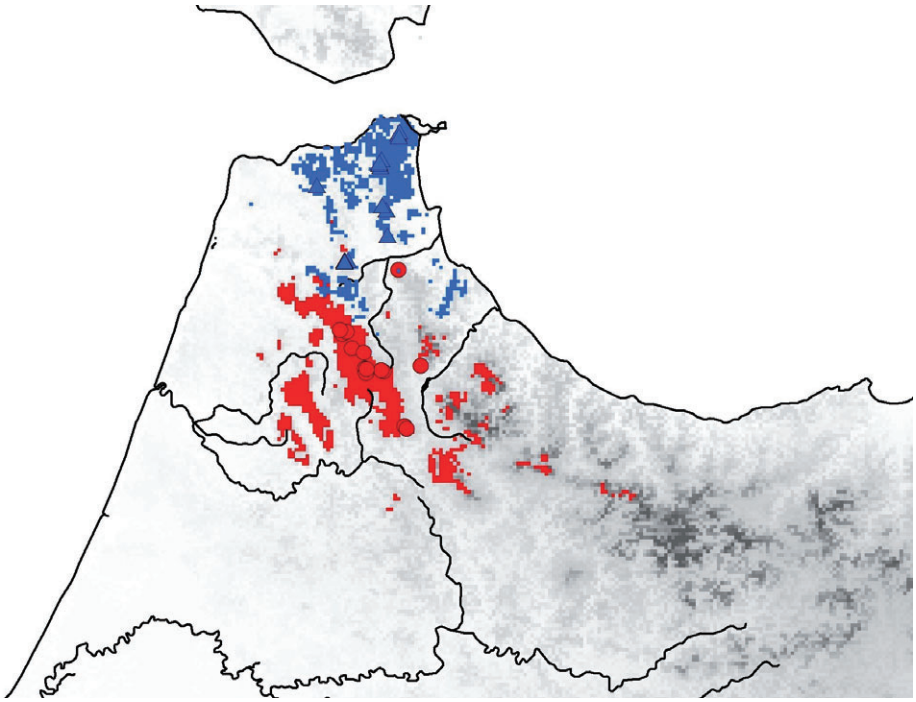

Figure 4. Predicted distribution of the viviparous (blue, based on coordinates displayed by triangles) and ovovivivparous (red, based on coordinates displayed by circles) populations of *Salamandra algira tingitana* above the average 10-percentile training threshold.

possible negative influence of spatial autocorrelated occurrence records, especially those originating from natural history museums or herbaria (e.g. Kaliontzopoulou *et al*., 2008; Phillips *et al*., 2009), it was concluded that 'models derived from precise presence records performed better than those inferred from published data of uncertain precision. Similarly, models inferred using all the data available, although clustered, performed better than those using reduced, randomly arranged data sets'. Because of the origin of the current data set we preferred to use all available occurrence records.

Post-Messinian divergence

The results presented herein and those of Steinfartz *et al*. (2000) and Escoriza *et al*. (2006) suggest that North African *Salamandra* most likely became separated from their European relatives after the opening of the Gibraltar land bridge at the end of the MSC. Two Maghrebian clades, separated by the Moulouya Basin in eastern Morocco, subsequently diverged (Steinfartz *et al*., 2000; Escoriza *et al*., 2006). This separation took place approximately 3.6 Mya, and seems to coincide with the beginning of a period of documented cyclic fluctuations in vegetation and climate in north-western Africa, which occurred between 3.7 and 1.7 Mya (Leroy & DuPont, 1994). During this period, vegetation fluctuated between more humid phases, characterized by tropical forests, and relatively drier phases, characterized by grasslands (Thompson & Fleming, 1996). The shift towards a colder and drier climate that occurred during the upper Pliocene (2.5–1.8 Mya; Webb & Bartlein, 1992) may have resulted in the isolation of *Salamandra* at increasing higher altitudes or other climatically favourable areas. This may have lead to the origin of *S. a. tingitana* towards the end of this period (approximately 1.6 Mya; Fig. 2), when it separated from populations of *S. a. algira* from the Rif and the Middle Atlas mountains. This is congruent with the fragmentation of other herpetofaunal species in the western Mediterranean Basin (see, for example, Carranza & Arnold, 2004; Zangari *et al*., 2006; Giovannotti *et al*., 2007; Stöck *et al*., 2008).

Separation of the first *S. a. tingitana* clade approximately 0.8 Mya during the Pleistocene roughly coincided with the separation between the Rif and Middle Atlas populations of *S. a. algira* and those of other montane Mediterranean caudates, such as *Salamandra atra pasubiensis* Bonato & Steinfartz, 2005 and *Salamandra atra aurorae* Trevisan, 1981 from *Salamandra atra atra* Laurenti, 1768, which are relatively young evolutionary lineages that now occur peripherally to their nominate taxa. Isolation of these latter taxa resulted from glacial-induced climatic

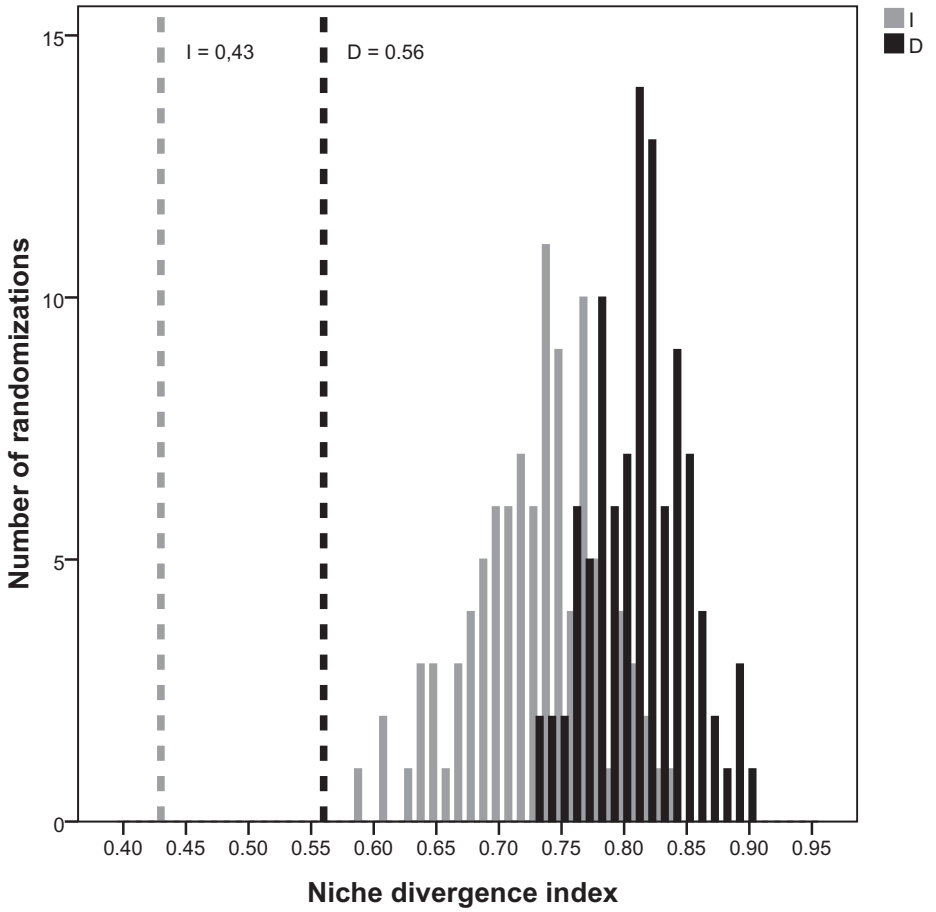

Figure 5. Degree of niche overlap for the *I* (light-grey) and *D* (black) niche similarity metrics created from MAXENT using 100 locality randomizations of the viviparous and ovoviviparous populations of *Salamandra algira tingitana*. The vertical dotted lines show the values observed for the actual data sets for MAXENT runs using all locality points (i.e. no test points).

oscillations (Bonato & Steinfartz, 2005), and the divergence displayed in *S. algira* also seems to follow this pattern. After the final split between populations of *S. a. tingitana* north and south of the Oued Martil occurred, approximately 0.6 Mya, the northern populations made the transition from ovoviviparity to viviparity. It is important to bear in mind that the information presented here just reflects the history of a single evolutionary unit (mtDNA). A definitive understanding of the evolution of North African *Salamandra* will not be possible until multiple informative nuclear markers are added to the mtDNA data.

Current environmental differentiation

Environmental differences between the distributions of the viviparous and ovoviviparous *S. a. tingitana* are mainly characterized by a higher dependence on precipitation in the latter, more stable and lower temperature oscillations in the the former, and the occurrence in different geology and vegetation types identified by the categorical variables. The climate and precipitation in the Tingitana Peninsula are highly influenced by both the proximity of the Atlantic Ocean and the Mediterranean Sea, and the altitude of the terrain (Santos, 1992). Orographic lift from the Atlantic (e.g. the Vendavel winds) causes a cooling effect and cloud formation, leading to precipitation on the western and central mountain ranges, which comprise the predicted distribution of the ovoviviparous *S. a. tingitana*. The maritime air of the Mediterranean, on the other hand, moves inland during autumn and winter, moderating seasonal cooling within the distribution range of the viviparous *S. a. tingitana*. This, together with the Levant winds that blow from the east during spring and summer, might be the cause of a more stable climate compared with the climate within the distribution range of the ovoviviparous populations. Additionally, viviparous *S.*

a. tingitana occur at relatively lower altitudes compared with the ovoviviparous (Fig. 1), which might result in a lower volume of precipitation because of a lack of orographic lift.

The two nonclimatic variables used to model each group, namely geology and NDVI, have been found to contribute considerably to the models (see Table 2). Although different by class, all geology types within the suitable predicted habitat of both groups consist of karstic limestone, which was already reported by Donaire-Barroso & Bogaerts (2003) to be highly important for the occurrence of North African *Salamandra*. As shown by Peterson & Nakazawa (2008), the inclusion of an NDVI can considerably improve model output, depending on the case, as has been proven for *Salamandra* (Omolo, 2006). As the NDVI values categorically increase towards that of the highest year-round green biomass, the presence of *S. a. tingitana* in the higher classes can be explained by the preference of *Salamandra* for forested habitats in North Africa (Bons & Geniez, 1996; Schleich, Kästle & Kabisch, 1996). Class 72 contributes more to the probability of the presence of the viviparous *S. a. tingitana* than class 75, which contributes more to the probability of presence of the ovoviviparous populations. Theoretically, this can be for two reasons: (1) the vegetation within the distribution of the viviparous populations is, on a mesoscale, notably different to that for the ovoviviparous populations, and/or (2) there is a larger area of bare soil present within class 72 compared with class 75. Evergreen oak species, which dominate the middle- and lowlands of the Tingitana peninsula, are replaced gradually at higher altitudes by pine trees (Ajbilou, Marañón & Arroyo, 2006), thus a higher volume of biomass would be expected at lower altitudes. The eastern Mediterranean area of the Tingitana peninsula, however, receives a smaller volume of rain compared with the western Atlantic-influenced mountains. This is mirrored by a gradient in vegetation communities (e.g. Sauvage, 1963; Moore *et al*., 1998). Additionally, viviparous *S. a. tingitana* are dependent on the karstic limestone formations, which are largely devoid of vegetation, and are therefore most likely identified as part of a lower class (class 72) because of their comparatively lower seasonal biomass with respect to the highest NDVI class 75.

The habitat differentiation interpreted by the model is mirrored by the fact that the predicted distributions of the two reproductive types do not overlap (Fig. 4). This is especially interesting considering the small scale of the study area and the degree of overlap displayed by other herpetofauna in similar studies (e.g. Martínez-Freiría *et al*., 2008). It does, however, illustrate the sudden environmental differences found within the distribution of *S. a. tingitana*.

Possible causes for the transition to viviparity

As suggested by Velo-Antón *et al*. (2007), the absence of surface water on karstic limestone soils can rapidly influence the transition of reproductive behaviour in *Salamandra* (within less than 10 000 years). There is little or no natural surface water north of the Oued Martil, and, if present, it is seldom used to deposit larvae (Donaire-Barroso & Bogaerts, 2003). Additionally, the lower average precipitation in the coldest quarter compared with that of the ovoviviparous populations compromises surface activity. As a result, *S. a. tingitana* north of the Oued Martil have to retreat into the karstic systems during unfavourable periods, which has also been suggested to influence the transition to viviparous reproduction in *Salamandra* (García-París *et al*., 2003b). An additional result of this cryptic life could be the relatively smaller size and ontogenetic tendency to hypoluteism of the viviparous *S. a. tingitana* (Donaire-Barroso & Bogaerts, 2003).

In conclusion, it is particularly interesting that the phenotype of the young, viviparous clade of *S. a. tingitana* seems to have diverged considerably from all other populations of *S. algira*, including the ovoviviparous populations of the same subspecies that live south of the Oued Martil, but also from the populations eastwards into the Rif and Middle Atlas mountains. All these populations of *S. algira* are ovoviviparous, morphologically conservative, and mainly differ in the presence or absence of red coloration (Bogaerts & Donaire-Barroso, 2003; Escoriza *et al*., 2006). This striking divergence, probably related to environmental pressures within the distribution of *S. a. tingitana*, again demonstrate the particularly high tendency for adaptive differentiation and speciation (*sensu* e.g. Steinfartz, Weitere & Tautz, 2007; Velo-Antón *et al*., 2007) within the genus *Salamandra.*

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REFERENCES

Ajbilou R, Marañón T, Arroyo J. 2006. Ecological and biogeographical analyses of Mediterranean forests of northern Morocco. *Acta Oecologica* **29:** 104–113.

Araújo MB, New N. 2007. Ensemble forecasting of species distributions. *Trends in Ecology and Evolution* **22:** 42–47.

Arntzen JW, García-París M. 1995. Morphological and allozyme studies of midwife toads (genus *Alytes*) including the description of two new taxa from Spain. *Contributions to Zoology* **65:** 5–34.

Austin MP. 1987. Models for the analysis of species' response to environmental gradients. *Vegetation* **69:** 35–45.

Batista V, Carranza S, Carretero MA, Harris DJ. 2006. Genetic variation within *Bufo viridis*: evidence from mitochondrial 12S and 16S rRNA DNA sequences. *Butlletí de la Societat Catalana d'Herpetologia* **17:** 24–33.

Belsley DA, Kuh E, Welsch RE. 1980. *Regression diagnostics: identifying influential data and sources of collinearity*. New York: John Wiley.

Bogaerts S, Donaire-Barroso D. 2003. Sobre el politipismo en *Salamandra algira* Bedriaga, 1883. *Boletín de la Asociación Herpetológica Española* **14:** 47–51.

Bonato L, Steinfartz S. 2005. Evolution of the melanistic colour in the Alpine salamander *Salamandra atra* as revealed by a new subspecies from the Venetian Prealps. *Italian Journal of Zoology* **72:** 253–260.

Bons J, Geniez P. 1996. *Amphibiens et Reptiles du Maroc (Sahara Occidental compris), Atlas biogéographique*. Barcelona: Asociación Herpetológica Española.

Capula M, Nascetti G, Lanza B, Bullini L, Crespo EG. 1985. Morphological and genetic differentiation between the Iberian and the other West Mediterranean *Discoglossus* species (Amphibia, Salientia, Discoglossidae). *Monitore Zoologico Italiano* **19:** 69–90.

Carranza S, Arnold EN. 2004. History of West Mediterranean newts, *Pleurodeles* (Amphibia: Salamandridae), inferred from old and recent DNA sequences. *Systematics and Biodiversity* **1:** 327–337.

Carranza S, Wade E. 2004. Taxonomic revision of Algero-Tunisian *Pleurodeles* (Caudata: Salamandridae) using molecular and morphological data. Revalidation of the taxon *Pleurodeles nebulosus* (Guichenot, 1850). *Zootaxa* **488:** 1–24.

Cunningham CW. 1997. Is congruence between data partitions a reliable predictor of phylogenetic accuracy? Empirically testing an iterative procedure for choosing among phylogenetic methods. *Systematic Biology* **46:** 464–478.

Donaire-Barroso D, Bogaerts S. 2001. Observations on viviparity of *Salamandra algira* in North Morocco. In: Lymberakis P, Valakos E, Pafilis P, Mylonas M, eds. *Herpetologica Candiana*. Natural History Museum of Crete: Irakleo, 147–151.

Donaire-Barroso D, Bogaerts S. 2003. A new subspecies of *Salamandra algira* Bedriaga, 1883 from northern Morocco. *Podarcis* **4:** 84–100.

Dubois A, Raffaëlli J. 2009. A new ergotaxonomy of the familiy Salamandridae Goldfuss, 1820 (Amphibia, Urodela). *Alytes* **26:** 1–85.

Elith J. 2002. Quantitative methods for modeling species habitat: comparative performance and an application to Australian plants. In: Ferson S, Burgman M, eds. *Quantitative methods for conservation biology*. New York: Springer, 39–58.

Elith J, Graham CH, Anderson RP *et al*. 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* **29:** 129–151.

Escoriza D, Comas MM. 2007. Description of a new subspecies of *Salamandra algira* Bedriaga, 1883 (Amphibia: Salamandridae) from the Beni Snassen massif (Northeast Moroco). *Salamandra* **43:** 77–90.

Escoriza D, Comas MM, Donaire D, Carranza S. 2006. Rediscovery of *Salamandra algira* Bedriaga, 1883 from the Beni Snassen massif (Morocco) and phylogenetic relationships of North African *Salamandra*. *Amphibia-Reptilia* **27:** 448–455.

Farris JS, Kallersjo M, Kluge AG, Bult C. 1994. Testing significance of incongruence. *Cladistics* **10:** 315–319.

García-París M, Buchholz DR, Parra-Olea G. 2003a. Phylogenetic relationships of Pelobatoidea re-examined using mtDNA. *Molecular Phylogenetics and Evolution* **28:** 12–23.

García-París M, Alcobendas M, Buckley D, Wake DB. 2003b. Dispersal of viviparity across contact zones in Iberian populations of fire salamanders (*Salamandra*) inferred from discordance of genetic and morphological traits. *Evolution* **57:** 129–143.

Gasser F, Joly JMJ. 1972. Existence d'un cycle sexuel biennal chez la femelle de *Salamandra salamandra fastuosa* Schreiber (Urodèle, Salamandridae) a différentes altitudes dans les Pyrénées centrales: influence des facteurs génétiques et climatiques. *Annales des Sciences Naturelles Zoologie et Biologie Animale* **14:** 427–444.

Giovannotti M, Cerioni PN, Kalboussi M, Aprea G, Caputo V. 2007. Phylogeographic Inferences from the mtDNA Variation of the Three-Toed Skink, *Chalcides chalcides* (Reptilia: Scincidae). *Journal of Experimental Zoology* **308B:** 297–307.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52:** 696–704.

Hernandez PA, Graham CH, Master LL, Albert DL. 2006. The effect of sample size and species characteristics on performance of different species distribution modelling methods. *Ecography* **29:** 773–785.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25:** 1965–1978.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

Jiménez-Valverde A, Lobo JM, Hortal J. 2008. Not as good as they seem: the importance of concepts in species distribution modelling. *Diversity and Distributions* **14:** 885–890.

Kaliontzopoulou A, Brito JC, Carretero MA, Larbes S, Harris DJ. 2008. Modelling the partially unknown distribution of wall lizards (*Podarcis*) in North Africa: ecological

affinities, potential areas of occurrence, and methodological constraints. *Canadian Journal of Zoology* **86:** 992–1001.

Khan MR, de Bie CAJM, van Keulen H, Smaling EMA, Real R. 2010. Disaggregating and mapping crop statistics using hypertemporal remote sensing. *International Journal of Applied Earth Observation and Geoinformation: JAG* **12:** 36–46.

Lalueza-Fox C, Castresana J, Samprieto L, Marquez-Bonet T, Alcover JA, Bertranpetit J. 2005. Molecular dating of caprines using ancient DNA sequences of *Myotragus balearicus*, an extinct endemic Balearic mammal. *BMC Evolutionary Biology* **5:** 70.

Leroy S, DuPont L. 1994. Development of vegetation and continental aridity in northwestern Africa during the Late Pliocene: the pollen record of ODP Site 658. *Palaeogeography, Palaeoclimatology, Palaeoecology* **109:** 295–316.

Martínez-Freiría F, Sillero N, Lizana M, Brito JC. 2008. GIS-based niche models identify environmental correlates sustaining a contact zone between three species of European vipers. *Diversity and Distributions* **14:** 452–461.

Martínez-Solano I, Gonçalves HA, Arntzen JW, García-París M. 2004. Phylogenetic relationships and biogeography of midwife toads (Discoglossidae: *Alytes*). *Journal of Biogeography* **31:** 603–618.

Mason-Gamer RJ, Kellogg EA. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Systematic Biology* **45:** 524–545.

Mattoccia M, Romano A, Sbordoni V. 2005. Mitochondrial DNA sequence analysis of the spectacled salamander, *Salamandrina terdigitata* (Urodela: Salamandridae), supports the existence of two distinct species. *Zootaxa* **995:** 1–19.

Michkevich MF, Farris JS. 1981. The implications of congruence in *Menidia*. *Systematic Zoology* **30:** 351–370.

Moore HM, Fox HR, Harrouni MC, El Alami A. 1998. Environmental challenges in the Rif mountains, northern Morocco. *Environmental Conservation* **25:** 354–365.

Omolo DP. 2006. Biodiversity patterns in changing Mediterranean landscapes: a modelling perspective. Unpublished Msc Thesis, International Institute for Geo-information Science and Earth Observation, Enschede, The Netherlands.

Pearson RG, Raxworthy CJ, Nakamura M, Townsend Peterson A. 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography* **34:** 102–117.

Peterson AT, Nakazawa Y. 2008. Environmental datasets matter in ecological niche modelling: an example with *Solenopsis invicta* and *Solenopsis richteri*. *Global Ecology and Biogeography* **17:** 135–144.

Phillips SJ, Dudík M. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography* **31:** 161–175.

Phillips SJ, Anderson RP, Shapire RE. 2006a. Maximum entropy modelling of species geographic distributions. *Ecological Modelling* **190:** 231–259.

Phillips SJ, Richardson K, Scachetti-Pereira R, Schapire RE, Soberón J, Williams S, Wisz MS, Zimmermann NE. 2006b. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* **29:** 129–151.

Phillips SJ, Dudík M, Elith J, Graham CH, Lehmann A, Leathwick J, Ferrier S. 2009. Sample selection bias and presence-only distribution models: implications for background and pseudo-absence data. *Ecological applications* **19:** 181–197.

Pleguezuelos JM, Fahd S, Carranza S. 2008. El papel del Estrecho de Gibraltar en la conformación de la actual fauna de anfibios y reptiles en el Mediterráneo Occidental. *Boletín de la Asociación Herpetológica Española* **19:** 1–16.

Posada D. 2008. jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

Sanderson MJ. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution* **14:** 1218–1231.

Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* **19:** 101–109.

Sanderson MJ, Doyle JA. 2001. Sources of error and confidence intervals in estimating the age of the angiosperm from rbcL and 18S rDNA data. *American Journal of Botany* **88:** 1499–1516.

Santos JW. 1992. *European theater weather orientation (ETWO)*. Germany: USAFE/DOW Headquarters US Air Forces in Europe.

Sauvage C. 1963. *Etages bioclimatiques. Notice et cartes au 1/2,000,000*. Atlas du Maroc Section II, planche 6b. Rabat: Comité Géographie Maroc.

Schleich H, Kästle W, Kabisch K. 1996. *Amphibians and reptiles of North Africa*. Koenigstein: Koeltz Scientific Books.

Steinfartz S, Veith M, Tautz D. 2000. Mitochondrial sequence analysis of *Salamandra* taxa suggests old splits of major lineages and postglacial recolonizations of Central Europe from distinct source populations of *Salamandra salamandra*. *Molecular Ecology* **9:** 397–410.

Steinfartz S, Weitere M, Tautz D. 2007. Tracing the first step to speciation: ecological and genetic differentiation of a salamander population in a small forest. *Molecular Ecology* **16:** 4550–4561.

Stöck M, Sicilia A, Belfiore NM, Buckley D, Lo Brutto S, Lo Valvo M, Arcuelo M. 2008. Post-Messinian evolutionary relationships across the Sicilian channel: Mitochondrial and nuclear markers link a new green toad from Sicily to African relatives. *BMC Evolutionary Biology* **8:** 56–74.

Thompson RS, Fleming RF. 1996. Middle Pliocene vegetation: reconstructions, paleoclimatic inferences, and boundary conditions for climate modeling. *Marine Micropaleontology* **27:** 27–49.

Thompson JD, Gibson TJ, Plewniak F, Jeanmourgin F, Higgins DG. 1997. The clustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24:** 4876–4882.

Velo-Antón G, García-París M, Galán P, Rivera AC. 2007. The evolution of viviparity in holocene islands: ecological adaptation versus phylogenetic descent along the transition from aquatic to terrestrial environments. *Journal of Zoological Systematics and Evolutionary Research* **45:** 345–352.

Warren DL, Glor RE, Turelli M. 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution* **62:** 2868–2883.

Warren DL, Glor RE, Turelli M. 2010. ENMTools: a toolbox for comparative studies of environmental niche models. *Ecography* **33:** 607-611.

Webb T, Bartlein PJ. 1992. Global changes during the last 3 million years: climatic 845 controls and biotic responses. *Annual Review of Ecology, Evolution, and Systematics* **23:** 141–173.

Wilcox TP, Derrick JZ, Heath TA, Hillis DM. 2002. Phylogenetic relationships of the dwarf boas and comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution* **25:** 361–371.

Wisz MS, Hijmans RJ, Li J, Peterson AT, Graham CH, Guisan A, NCEAS Predicting Species Distributions Working Group. 2008. Effects of sample size on the performance of species distribution models. *Diversity and Distributions* **14:** 763–773.

Zangari F, Cimmaruta R, Nascetti G. 2006. Genetic relationships of the western Mediterranean painted frogs based on allozymes and mitochondrial markers: evolutionary and taxonomic inferences (Amphibia, Anura, Discoglossidae). *Biological Journal of the Linnean Society* **87:** 515–536.

APPENDIX 1

Uncorrected genetic distances (p-distance) in % for the mitochondrial gene cyt*b* (A) and *12S* rRNA (B) between different populations of *S. algira* from Morocco. 1, *Salamandra algira tingitana* (ovoviviparous population), specimens 4–5 and 11–16 from Table 1; 2, *S. a. tingitana* (viviparous population), specimens 6–10 from Table 1; 3, *Salamandra algira algira* (Rif Mountains), specimens 24–36 from Table 1; 4, *S. a. algira* (Atlas Mountains), specimens 17–23 from Table 1; 5, *Salamandra algira spelaea* (Beni Snassen), specimens 1–3 from Table 1. Numbers in the lower left diagonal (in bold) are the genetic distances, and numbers in the upper right diagonal are the associated standard errors. The column labelled as 'within' represents the level of genetic variability within each one of the five groups.

A

	1	2	3	4	5	Within
1	–	0.50	0.95	1.21	1.60	0.73
2	**1.15**	–	1.03	1.27	1.57	0.49
3	**2.95**	**3.03**	–	0.93	1.68	0.23
4	**4.48**	**4.56**	**2.54**	–	1.68	0.31
5	**7.12**	**6.61**	**7.56**	**7.37**	–	0.00

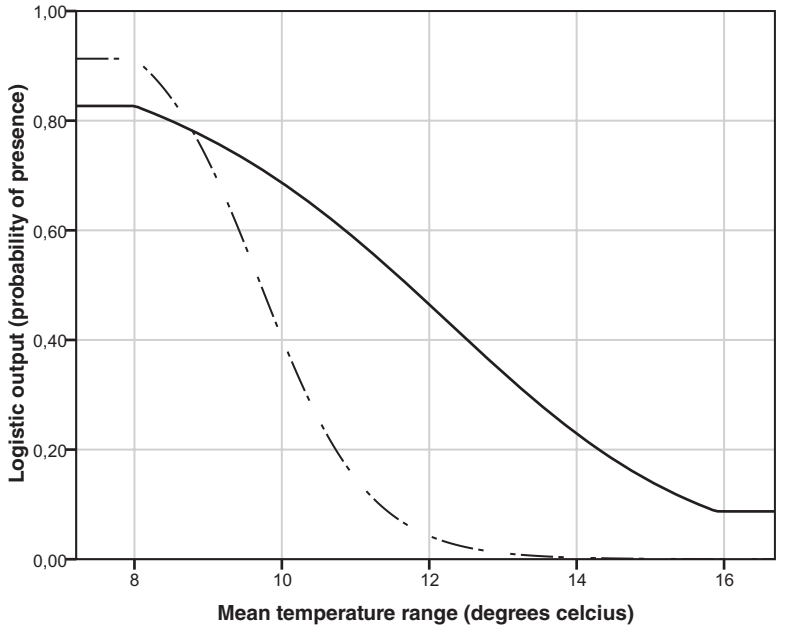
B

	1	2	3	4	5	Within
1	–	0.36	0.48	0.46	0.86	0.32
2	**0.77**	–	0.52	0.50	0.87	0.29
3	**1.01**	**1.13**	–	0.0.45	0.87	0.16
4	**1.09**	**1.20**	**0.85**	–	0.85	0.31
5	**3.00**	**2.96**	**2.69**	**2.84**	–	0.00

APPENDIX 2

Response curves for the major continuous predictor variables of the ovoviviparous (solid line) and viviparous (dashed line) populations of *Salamandra algira tingitana*.

a



b