



Evolution of morphology in Western Palaeartic salamanders: do climate and reproductive mode drive body size and shape in terrestrial salamanders?

FÈLIX AMAT

Àrea d'Herpetologia, BiBIO, Museu de Granollers – Ciències Naturals. Palaudàries 102, Granollers 08402, Catalonia, Spain

felixamat09@gmail.com

Manuscript received: 23 March 2020

Accepted: 2 October 2020 by ALEXANDER KUPFER

Abstract. Fully terrestrial lifestyles have evolved in a lineage of salamanders living since the Oligocene in the Western Palaeartic that adaptively radiated in morphology, ecology and reproductive modes. I analyse the relationships between body size and shape, and climate and reproductive mode in true terrestrial salamanders of the family Salamandridae. Principal component analyses were carried out on bioclimatic and morphologic data and used to reconstruct the diversification of these along the evolutionary timeline of this lineage. Additionally, a phylogenetic generalized least squares regression was performed in order to test the effect of climate and reproductive mode on their morphologic diversification. Western Palaeartic salamanders experienced a strong disparity between small and elongated forms early during their evolution, and short-bodied species exhibit large size variation. Body elongation in the Salamandridae seems to be linked to an adaptation to semifossorial habits and is currently restricted to a narrow range of climatic conditions, which is in contrast to the other terrestrial species. Within this latter group, large species exclusively live at the southernmost edge of the geographic range of true terrestrial salamanders. Thus, Bergmann's rule is not adhered to by true salamanders, since larger species live in hot climates. The ancestral oviparous mode of reproduction in the Salamandridae could be maintained in elongated salamanders due to their body shapes not posing constraints, whereas the largest sized short-bodied species have developed larviparism.

Key words. Morphology, evolution, Salamandridae, ecological diversification, climatic niche.

Introduction

Based on molecular evidence and fossil records, Salamandrids appeared 113–55 Mya (ESTES 1981, ZHANG et al. 2008) and diversified into 105 species distributed in North America, Europe and temperate, but also tropical, regions of Asia. The ecological radiation of this family involved diversifications of body shape, tongue morphology, reproductive strategies, and courtship behaviour (e.g., ÖZETI & WAKE 1969, WAKE & ÖZETI 1969, HOUCK & ARNOLD 2003). Remarkably, one salamandrid lineage has developed several morphological and reproductive innovations in order to shed its dependence on water, leading to truly terrestrial lifestyles in some species (MILLER & LARSEN 1990, WAKE 2004). Named “true” salamanders (VEITH et al. 1998), they live in the western parts of the Palaeartic and are represented by the genera *Chioglossa*, *Mertensiella*, *Salamandra* and *Lyciasalamandra*. Phylogenetic evidence suggests an Oligocene origin of the Western Palaeartic salamanders that could be placed in the Near East (RODRÍGUEZ et al. 2017), where *Mertensiella*, *Lyciasalamandra*, and one basal lineage of *Salamandra*, *S. infraimmaculata* live (EHL et al. 2019). Since that period, they have experienced diversifications of body size, shape and coloration, colonizing a wide

variety of climates ranging from that of Mediterranean islands to that of Alpine mountain ranges. However, only the species of *Lyciasalamandra* have become totally decoupled from water by developing not only terrestrial reproduction, but also viviparity, even supporting it with matrotrophy (HÄFELI 1971, BUCKLEY et al. 2009). Accordingly, it is likely that in Western Palaeartic salamanders, ecology, body size and shape evolved driven by the disparity of climatic environments and the reproductive strategies developing in this lineage.

Correlations between the evolution of body shape and other biological traits, or even abiotic ones, has been reported by several researchers and is considered evidence of adaptation (e.g., GRIZANTE et al. 2012, BONETT & BLAIR 2017). The main aim of this study is to characterize the extent of body size and shape diversity in true salamanders, how it evolved through time, and test whether current climatic conditions and reproductive modes may have acted as drivers of their morphologic diversification.

Material and methods

A sample of 768 specimens of Western Palaeartic salamanders, comprising all species of *Mertensiella*, *Salamandra*,

Chioglossa, and most of *Lyciasalamandra* (Fig. 1) from collections housed at natural history museums was analyzed (Supplementary document S1). In order to capture the variation of body size and shape, seven variables were taken: length from the tip of the snout to the posterior margin of the cloacal protuberance (SVL), head length and maximum width, forelimb, hindlimb and tail length, and inter-limb distance measured between the closest point of insertion of extremities on the right ventral side (Table 1). Despite the sexual dimorphism in some traits (REINHARD et al. 2015), differences between lineages of terrestrial salamanders are likely more phylogenetically informative than those between sexes. Therefore and in order to maximize sample sizes, males and females were pooled for the analysis. Bioclimatic data on each taxon were extracted at 30 arc-second intervals (7182 records) from the Worldclimate database (FICK & HIJMANS 2017), using gathered data on their distribution (Herpnet, Inaturalist, and other sources: Supplementary document S2). The following variables representing climatic trends and extreme variations were selected: mean annual temperature, maximum temperature of warmest month, minimum temperature of coldest month, annual precipitation, precipitation of wettest quarter, and precipitation of driest quarter. Principal component analyses (PCAs) of morphometric and bioclimatic data sets were performed using the mean scores for each salamander taxon with the aid of Statistica 4.5. In order to test whether morphological and climatic variables are labile or strongly shaped by the evolutionary history, the phylogenetic signal was checked

using the K and Lambda tests (PAGEL 1999, BLOMBERG et al. 2003) incorporated in the function *phylosig* in *Phytools* 0.6 (REVELL 2012) using R 3.6.2. A phylogenetic tree of Western Palearctic terrestrial salamanders was built using available information about their evolutionary relationships (RODRÍGUEZ et al. 2017, EHL et al. 2019). Branch lengths were estimated by mean maximum likelihoods upon the HKY-86 model with invariant sites and gamma modelling of variable sites using a dataset formed by 12sRNA and cytochrome *b* mitochondrial genes (Supplementary document S3). Branch lengths were ultrametrized by applying a molecular clock based on three external points of calibration (Supplementary document S4) applying the *RelTime* method (TAMURA et al. 2012). All these analyses were performed by means of *Mega* 7.0 (KUMAR et al. 2016).

Evolution of morphologic and climatic PCA was traced by means of character reconstructions based on maximum likelihoods using the function *fastAnc* incorporated in *Phytools* 0.6 and the ultrametric phylogenetic tree. To test whether climate and reproductive mode affected body size and shape diversification, a phylogenetic generalized least squares (PGLS) regression was performed. Reproductive mode was defined by means of three categorical variables, oviparity, larviparity and pueriparity, coding the absence or presence of a trait as 0 or 1, which facilitated the inclusion of taxa with interpopulation variation (i.e., *Salamanca salamandra gallaica*, *S. s. bernardezi*). Analyses were conducted using *Phytools* 0.6 and non-phylogenetic linear-squared regressions on R 3.6.2.



Figure 1. Geographic distribution of the four genera of Western Palearctic salamanders: monotypic and elongated *Chioglossa* (salmon) and *Mertensiella* (orange), and short-bodied *Lyciasalamandra* (black) and *Salamanca* (blue). The latter genus is geographically the most widespread of the true salamanders.

Table 1. Means and standard deviations of morphometric variables of *Mertensiella*, *Chioglossa*, *Lyciasalamandra* and *Salamandra*.

Taxa	N	SVL	Head length	Head width	Limb interval	Forelimb length	Hindlimb length	Tail length
<i>Chioglossa lusitanica</i>	5	48.5±0.7	9.4±0.3	5.6±0.4	25.0±1.6	9.5±5.2	12.6±0.4	86.0±8.1
<i>Mertensiella caucasica</i>	8	62.5±5.3	14.4±1.1	9.1±0.6	30.7±4.0	16.3±0.7	19.6±1.0	84.7±14.2
<i>Lyciasalamandra fazilae</i>	10	60.0±7.9	14.0±1.4	10.4±1.1	27.0±4.2	17.8±2.7	20.6±2.3	47.7±7.7
<i>Lyciasalamandra luschni</i>	10	66.9±4.0	14.9±0.9	10.8±0.7	31.4±4.4	19.1±1.1	22.0±1.6	48.6±4.3
<i>Lyciasalamandra atifi</i>	2	74.9±13.3	17.3±1.6	12.0±1.9	35.4±6.9	21.6±2.7	25.2±2.1	64.5±12.0
<i>Lyciasalamandra billae</i>	2	67.9±1.5	14.9±0.1	10.6±0.2	30.7±1.9	17.6±1.2	21.7±2.0	53.4±2.7
<i>Lyciasalamandra flavimembris</i>	2	66.7±0.4	15.0±0.4	10.6±0.2	31.7±0.6	18.8±3.1	21.5±0.7	52.9±3.3
<i>Salamandra atra</i>	27	76.3±15.2	17.3±3.1	12.0±2.4	35.2±8.9	20.4±4.9	23.6±4.5	50.4±11.0
<i>Salamandra corsica</i>	18	100.8±8.3	23.3±2.1	18.3±1.4	44.0±4.9	29.4±3.8	32.9±3.9	60.0±8.2
<i>Salamandra i. infraimmaculata</i>	8	128.2±22.2	26.6±4.0	21.3±3.1	60.5±12.0	33.4±6.2	38.1±7.9	82.8±14.8
<i>Salamandra lanzai</i>	9	83.7±5.0	20.4±1.0	14.3±0.7	37.6±2.9	21.6±1.3	25.5±1.4	58.3±6.1
<i>Salamandra a. algira</i>	2	97.9±1.5	22.4±0.4	15.8±0.3	47.3±3.8	27.1±1.3	29.8±0.2	64.7±5.8
<i>Salamandra a. splendens</i>	3	74.6±4.9	17.4±0.9	11.9±0.6	35.6±3.1	19.3±0.4	22.2±2.2	55.9±2.9
<i>Salamandra a. spelaea</i>	15	89.3±16.5	20.3±2.6	14.5±1.8	42.1±10.1	23.7±4.7	27.5±5.5	63.2±17.4
<i>Salamandra a. tingitana</i>	12	95.3±9.3	21.0±1.6	15.2±1.1	44.6±4.7	25.2±2.5	30.6±3.9	71.5±8.6
<i>Salamandra a. atra</i>	27	76.3±15.8	17.3±3.1	12.0±2.5	35.0±9.1	20.4±5.0	23.6±4.6	50.7±11.1
<i>Salamandra s. almanzorisi</i>	75	77.0±7.1	18.4±2.1	14.8±1.5	33.2±4.5	21.4±2.7	24.4±2.7	49.4±6.0
<i>Salamandra s. bejarae</i>	48	97.1±8.2	21.8±2.2	16.7±1.5	43.3±5.4	22.7±3.1	30.9±2.9	59.7±8.2
<i>Salamandra s. bernardezi</i>	27	75.4±9.7	16.6±1.7	12.1±1.6	33.1±5.0	19.2±2.8	22.9±2.7	47.8±6.6
<i>Salamandra s. crespoidi</i>	2	91.6±6.0	22.0±0.9	15.7±0.7	42.0±5.0	24.8±3.1	30.9±0.9	55.6±3.6
<i>Salamandra s. fastuosa</i>	113	82.6±10.3	18.1±2.4	13.7±1.8	35.3±6.1	22.4±3.2	26.0±3.7	52.4±8.3
<i>Salamandra s. gallaica</i>	81	98.5±10.1	22.4±2.1	17.3±1.8	42.7±6.1	28.0±3.6	32.1±3.8	59.7±8.1
<i>Salamandra s. giglioli</i>	40	85.9±6.4	19.6±1.6	14.8±1.2	37.3±3.9	24.1±2.4	27.2±2.6	54.6±6.4
<i>Salamandra s. longirostris</i>	36	110.2±9.7	24.2±2.6	18.0±1.5	51.9±6.7	28.9±3.5	34.1±3.5	64.9±7.9
<i>Salamandra s. morrenica</i>	52	103.0±8.9	23.2±2.0	17.0±1.9	47.2±4.9	28.8±3.6	32.9±3.9	61.7±8.7
<i>Salamandra s. salamandra</i>	9	95.2±10.7	21.9±2.5	16.1±2.1	43.9±7.0	26.0±3.0	29.9±3.3	62.5±8.3
<i>Salamandra s. terrestris</i>	120	95.1±9.6	21.2±2.0	15.8±1.6	43.2±6.2	25.6±2.6	30.0±3.2	58.7±7.5

Results

Body size diversified during the evolution of terrestrial salamandrids and currently ranges from the smallest species *Chioglossa lusitanica* (maximum SVL 49.2 mm) to the largest, *Salamandra infraimmaculata* (max. SVL 150.4 mm, Table 1). Body size and shape diversifications were well represented by the PCAs, accounting for a larger portion of variation of the seven morphometric variables (Table 2). The first PC accounted for 82.8% of the total variation and displayed a typical size structure in the shape of high and negative coefficients in all the morphometric variables. The second PC was polarized by the negative coefficients of tail length and inter-limb distance, and on the other hand by the extreme positive coefficients of head width and forelimb length. Thus, this second component may be likely associated with body shape diversity. Western Palearctic terrestrial salamanders displayed two contrasting patterns of body shape (Fig. 2a). *Chioglossa* and *Mertensiella* are two closely related small-bodied lineages characterized by slim and elongated bodies, tails and narrow heads, a trend more developed in the North Western Iberian than in the Cau-

Table 2. PCA loadings of mean values in the seven linear-morphometric variables covering the morphological diversification of Western Palearctic salamanders.

Variable	1 st PC	2 nd PC
SVL	-0.993	-0.059
Head length	-0.988	0.017
Head width	-0.982	0.137
Limb interval	-0.956	-0.163
Forelimb length	-0.978	0.136
Hindlimb length	-0.991	0.066
Tail length	-0.138	-0.987
Eigenvalues	5.802	1.048
% of explained variation	82.886	97.863

casian endemics. Body shape in *Lyciasalamandra* and *Salamandra* was roughly similar, and the main trait explaining interspecific variation was overall SVL. Thus, *Lyciasalamandra* was turned up as a genus of uniformly small-sized salamanders grouped together with *Salamandra atra* and

the subspecies *bernardezi*, *giglioli* and *almanzorisi* of *S. salamandra*, all of which are small-sized as well. At the opposite end, the Middle Eastern *Salamandra infraimmaculata* and the North African *S. algira* have the largest SVLs, followed by the southern Iberian subspecies *S. s. longirostris*. Based on their placement in the second PC, *Salamandra infraimmaculata* exhibited a slight trend towards body elongation.

Bioclimatic variables selected for the analyses reflected the diversity of environmental conditions experienced by Western Palearctic salamanders. The first PC explained a total variation of 60.5% (Table 3), displaying an inverted influence of temperature vs. precipitation on the first PC. The second PC produced high negative coefficients for annual precipitation and maximum precipitation in contrast to the positively weighted minimum precipitation. The harshest environmental conditions (i.e., lower precipitation and high temperatures) were found in the Middle East, which is inhabited by *Lyciasalamandra* and *Salamandra infraimmaculata* and, to a lesser extent, by *Salamandra algira* (Fig. 2b). Interestingly, the two elongated salamander genera *Chioglossa* and *Mertensiella* exhibited close affinities to climates characterized by buffered temperatures and high amounts of precipitation. At the other extreme end of the climatic variation experienced by true salamanders, cold and rainy climates were characteristic of the habitats of the Alpine species *Salamandra lanzai* and *S. atra*.

Body size and shape diversifications in *Mertensiella*, *Chioglossa*, *Lyciasalamandra* and *Salamandra* were influenced by the evolutionary history of this grouping, although the strength of the phylogenetic effect strongly differs between methods of testing. The Lambda test suggested strong phylogenetic constraints on the evolution of size

Table 3. PCA performed on the six selected bioclimatic variables along the distribution of *Mertensiella*, *Chioglossa*, *Lyciasalamandra* and *Salamandra*.

Variable	1 st PC	2 nd PC
Mean annual temperature	0.964	-0.132
Max temperature of warmest month	0.967	-0.015
Min temperature of coldest month	0.875	-0.019
Annual precipitation	-0.459	-0.871
Precipitation of wettest quarter	0.203	-0.964
Precipitation of driest quarter	-0.865	0.051
Eigenvalues	3.633	1.710
% of explained variation	60.566	89.076

and shape (first PC: $\lambda=0.867$, $P<0.001$; second PC: $\lambda=0.922$, $P<0.001$) while the K test turned up a weak effect (first PC: $K=0.014$, $P=0.028$; second PC: $K=0.029$, $P=0.011$). Similar results were obtained in the case of the first PC extracted from bioclimatic variables ($\lambda=0.966$, $P<0.001$; $K=0.015$, $P=0.037$), but not in the second PC ($\lambda=0.241$, $P=0.423$; $K=0.002$, $P=0.400$). Therefore, only non-phylogenetic linear-squared regressions were performed on this PC.

The ancestral condition of terrestrial Western Palearctic salamandrids was not that of an elongated body (Fig. 3a), which was first developed by the common ancestor of *Chioglossa* and *Mertensiella*. Based on maximum likelihood reconstruction, the common ancestor of this group was a small-sized and short-bodied species (Fig. 3b). From this ancestral condition, SVL slightly increased in the common ancestor of *Lyciasalamandra* and *Salamandra*. The former

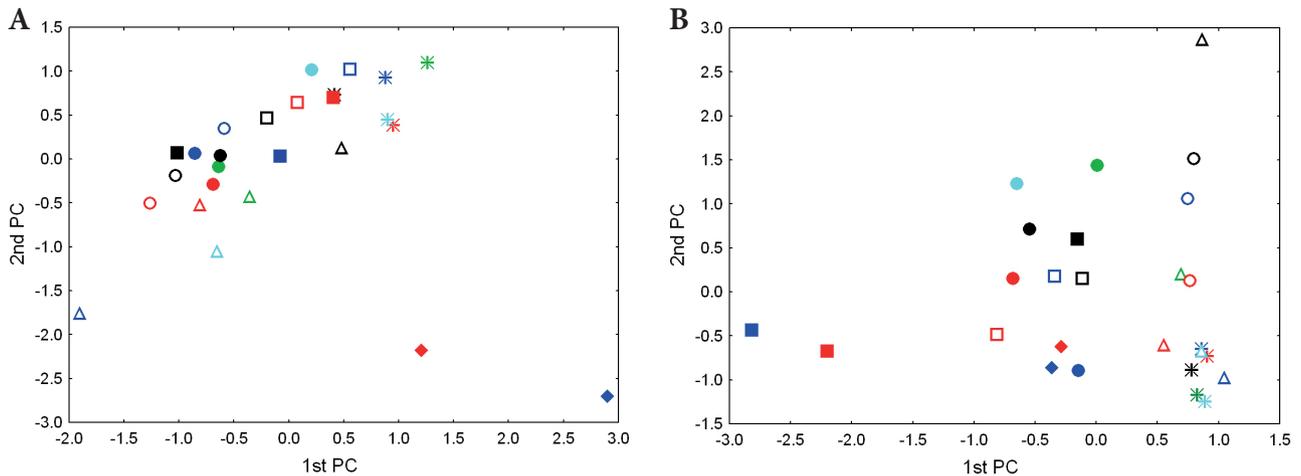


Figure 2. Morphological and climatic diversification in terrestrial Western Palearctic salamanders: A) Morphospace generated by the two first PCs extracted from seven linear-morphometric measurements. B) Range of variation of climatic conditions within the range of terrestrial Western Palearctic salamanders as represented by the two first PCs extracted from the six bioclimatic variables. Taxa are symbolized as: full rhombs, *Chioglossa lusitanica* (blue), *Mertensiella caucasica* (red); asterisks, *L. atifi* (black), *L. billae* (red), *L. fazilae* (green), *L. flavimembris* (cyan), *L. luschani* (blue); empty triangles, *Salamandra algira algira* (red), *S. a. splendens* (black), *S. a. tingitana* (cyan), *S. a. spelaea* (green), *Salamandra infraimmaculata* (blue); full squares, *Salamandra atra* (red), *S. lanzai* (blue), *S. corsica* (black); full circles, *Salamandra s. almanzorisi* (cyan), *S. s. salamandra* (red), *S. s. terrestris* (black), *S. s. bejarae* (green), *S. s. gallaica* (blue); empty squares, *S. s. bernardezi* (blue), *S. s. fastuosa* (red), *S. s. giglioli* (black); empty circles, *S. s. crespoi* (blue), *S. s. longirostris* (red), *S. s. morenica* (black).

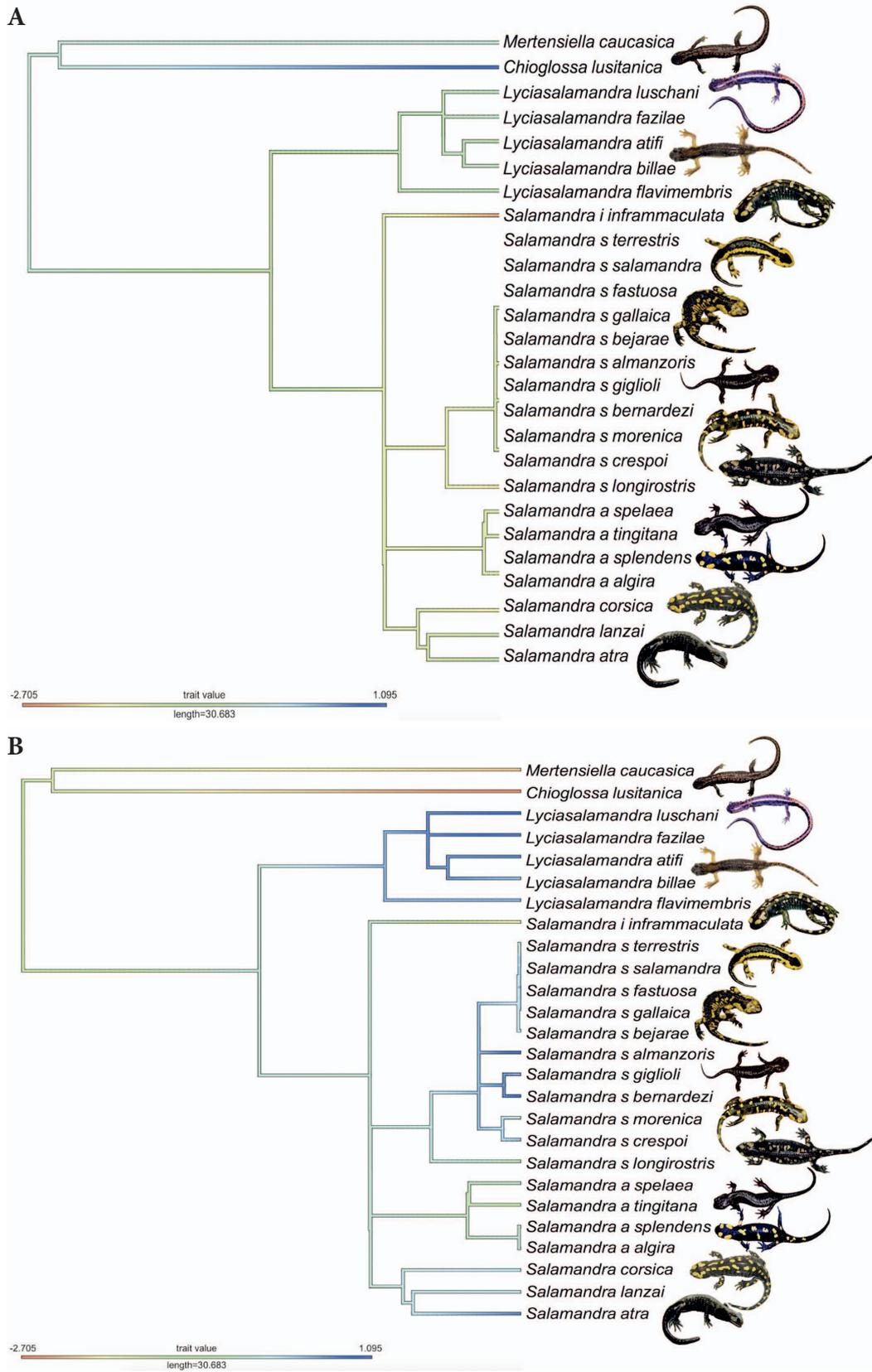


Figure 3. Maximum likelihood reconstruction of the evolution of morphology based on the first PC (a) and second PC (b) in terrestrial Western Palearctic salamanders.

genus then retained this medium size while *Salamandra* experienced further body length diversification. Large salamanders independently appeared several times during the evolutionary history of this genus, i.e., in *Salamandra corsica*, *S. s. longirostris*, the *crespoi-morenica* lineage, and today's largest species, *S. inframaculata*. Conversely, size reduction evolved in the Ibero-south Italian lineage of *Salamandra salamandra*, as represented today by *S. s. almanzoris*, *giglioli* and *bernardezi*, and the Alpine clade of *S. atra* and *S. lanzai*. Maximum likelihood reconstruction indicates that the original climatic conditions in which the common ancestor of *Salamandra*, *Lyciasalamandra*, *Chioglossa* and *Mertensiella* evolved were similar to those currently experienced by these two small and elongated latter species (Fig. 4). Adaptation to xeric environments occurred independently five times during the evolution of the group (in *Lyciasalamandra* and four lineages of *Salamandra*) while extreme cold habitats were colonized only once during the evolution of the Alpine lineage.

The evolution of body size and shape in Western Palearctic terrestrial salamanders was found to be related with the climatic conditions. PGLS regression revealed that first PC extracted from the six bioclimatic variables was significantly related with size and shape PCs (Table 4). The relationship was positive with the first morphologic PC, revealing that SVL increased with rising temperature and decreased with rising precipitation. The opposite pattern

was found in the second PC, indicating that body elongation was related with buffered precipitation and temperature during the driest and coldest months. Linear-squared regression of morphologic PCs on the second climatic PC was not significant in any of the tested scenarios (Table 4), congruent with the low percentage of variation accounted for by this component. PGLS regression exhibited some reproductive modes to have a significant effect of on body size and shape (Table 5). Thus, larviparous salamanders tend to be largest, but the other modes did not show a significant relationship with body size, and oviparity is related with body elongation.

Discussion

The wide array of climatic conditions experienced by Western Palearctic salamanders during their long evolutionary history (EHL et al. 2019) may have driven their diversification in body shape and size, as my results suggest. Early during their evolution, true salamanders strongly diverged into elongated and short-bodied forms probably as a result of their ecological divergence in a quest to exploit different niches. *Chioglossa* and *Mertensiella* thus developed semi-fossorial habits and now live in microhabitats with high moisture levels generated by the proximity of fast-flowing streams in mountain areas (TARKHNISHVILI 1993,

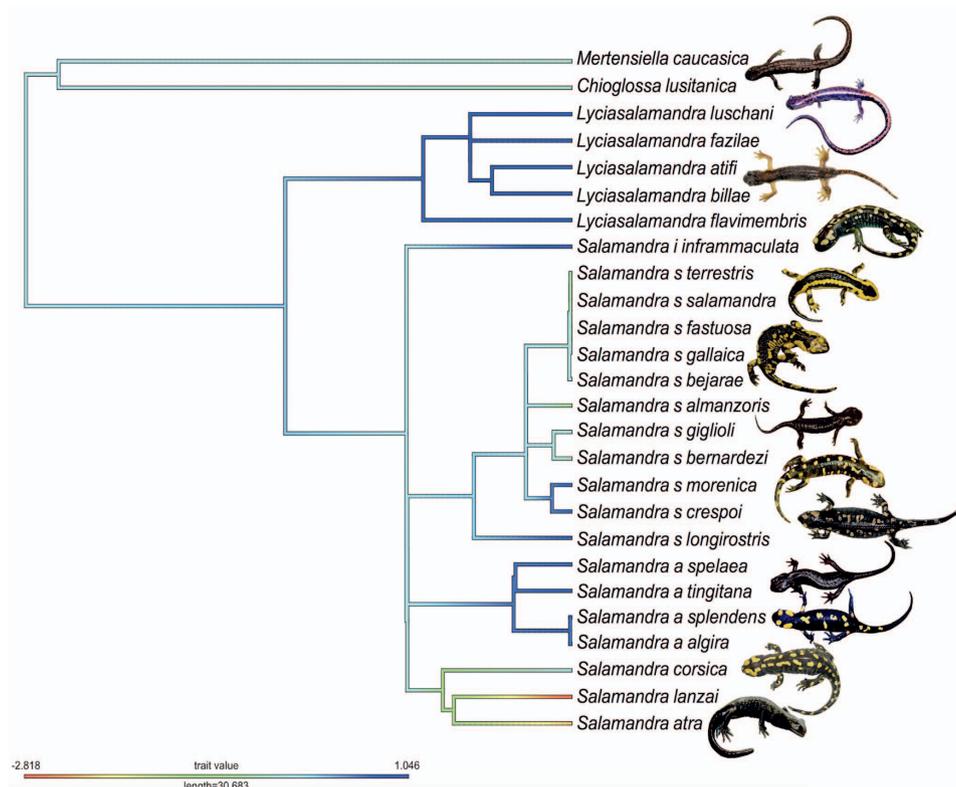


Figure 4. Maximum likelihood reconstruction of the evolution of first principal bioclimatic component among terrestrial Western Palearctic salamanders.

Table 4. Relationships between bioclimatic (CPC) and morphometric (MPC) principal components of Western Palearctic salamanders assessed by PLGS and OLS regressions. Significant p-values are given in bold.

Predictor	Predicted	Slope \pm SE	T	P	Intercept \pm SE	T	P	R ²
1 st CPC	1 st MPC	0.048 \pm 0.020	2.357	0.029	0.001 \pm 0.0020	0.001	0.999	0.235
	2 nd MPC	-0.050 \pm 0.205	-2.478	0.023	0.001 \pm 0.019	0.001	1.000	0.254
2 nd CPC	1 st MPC	-0.025 \pm 0.019	-1.324	0.197	0.001 \pm 0.0189	0.001	1.000	0.065
	2 nd MPC	0.019 \pm 0.019	0.995	0.329	-0.001 \pm 0.019	0.001	1.000	0.038

Table 5. Relationships between morphometric (MPC) principal components and reproductive mode of Western Palearctic salamanders assessed by PLGS regression.

Predicted	Predictor	Slope \pm SE	T	P	Intercept \pm SE	T	P	R ²
1 st MPC	Oviparity	2.011 \pm 1.062	1.892	0.070	0.040 \pm 1.105	0.036	0.970	0.129
	Larviparity	-1.346 \pm 0.439	-3.061	0.005	1.321 \pm 0.839	1.574	0.128	0.280
	Puelparity	0.464 \pm 0.270	1.721	0.098	0.920 \pm 1.151	0.799	0.431	0.109
2 nd MPC	Oviparity	-2.610 \pm 1.007	-2.589	0.016	0.168 \pm 1.065	0.157	0.875	0.218
	Larviparity	-0.583 \pm 0.559	-1.042	0.307	-1.087 \pm 1.283	-0.847	0.405	0.043
	Puelparity	0.415 \pm 0.211	1.959	0.061	-1.348 \pm 1.308	-1.030	0.313	0.137

ARNTZEN 1995). Body elongation could be an adaptation to this semi-fossorial lifestyle, as it is also found in other salamanders with similar lifestyles (for example, *Batrachoseps* or *Oedipina*, PARRA-OLEA & WAKE 2001), but at the same time it may impose strong physiological constraints. Elongated bodies have a greater body surface/volume ratio (BAKEN et al. 2019) that will increase the loss of water, which is dramatically important in species that breathe via their skin. Hence, elongated Salamandridae are inevitably restricted to very humid microhabitats. However, our analyses showed that the first climatic PC was influenced by the phylogeny and a strong climatic similarity between *Chioglossa* and *Mertensiella*. Fossil and biogeographic evidence indicates that the ancestor of these elongated salamanders had a large geographic distribution in the past, becoming restricted to climatic conditions currently found only in northwestern Iberia and the south of Caucasus (BLAIN et al. 2009, EHL et al. 2019). Hence, this climatic affinity between the two species could be the result of phylogenetic conservatism (WIENS & GRAHAM 2005). In contrast, *Lyciasalamandra* and *Salamandra* have shorter bodies and live on the floor, mainly in forested habitats. In order to avoid the risk of desiccation they have developed almost strictly nocturnal activity patterns during rainy or otherwise humid nights.

Remarkably, my analyses revealed that Western Palearctic salamanders defy Bergmann's rule (WATT et al. 2010) and exhibit an opposing trend. Very limited evidence for the validity of this rule has been found in amphibians and in salamanders in particular (ASHTON 2002, ADAMS et al. 2008). Likely, true salamanders deviate from this rule because the largest salamander species (*Salamandra algira* and *S. infraimmaculata*) happen to inhabit the warmer and xeric areas at the southernmost margins of the overall dis-

tribution range. Even though high-altitude taxa such as, for example, *Salamandra atra* and *S. s. almanzoris*, are smaller, and the largest member of the Alpine clade, *S. lanzai*, lives at the highest altitudes in the coldest climates (ANDREONE & SINDACO 1989).

The harsh climatic conditions faced by salamanders at the southernmost edge of the lineage distribution could force them to strongly reduce their annual activity (DEGANI 1996), thus producing delayed sexual maturity, consequently facilitating a longer period of subadult growth, and therefore leading to larger sizes. Nevertheless, the delay in reaching sexual maturity is not so large as to explain the differences in SVL between large and small species (SINSCH et al. 2017, ALTUNIŞIK 2018), thus suggesting a more complex background. Perhaps large salamanders can adapt better to dry habitats by having a lower ratio between body surface and volume.

Viviparity is one remarkable evolutionary process to adapt to the truly terrestrial lifestyle that is today found among urodeles only in Western Palearctic salamanders (BUCKLEY et al. 2007). Within this group, oviparity only occurs in the elongated *Mertensiella* and *Chioglossa* spp., while the short-bodied *Lyciasalamandra* and *Salamandra* spp. have larviparous and pueriparous modes of reproduction (BUCKLEY et al. 2009). Thus, it is likely that the development of small and elongated bodies in *Chioglossa* and *Mertensiella* has enforced the retention of the ancestral oviparous mode of reproduction by not providing enough abdominal space for bearing larvae. Despite this effect of body size on the trade-off between clutch and offspring size (SALTHER 1969), my results provide very limited support for a clear relationship with larviparous or pueriparous reproduction. Species and subspecies at the southern edge of the geographic area inhabited by *Salamandra* are those with

the largest sizes and are mainly larviparous, but the northernmost distributed *Salamandra algira tingitana* also gives birth to numerous fully developed young (DONAIRE et al. 2001). Pueriparity is obligatory in the Mediterranean and small-sized *Lyciasalamandra* (OZETI 1979), but it is also the mode of reproduction of the Alpine salamanders and some populations of *Salamandra Salamandra*, namely the subspecies *gallaica*, *bernardezi* and *fastuosa* (GARCÍA-PARÍS et al. 2003, VELO-ANTÓN et al. 2012). The development of the variety of derived reproductive modes, from larviparity to pueriparity with oophagy, adelphophagy or matrotrophy, seem to constitute very complex phenomena driven by factors other than body size (DOPAZO & KORENBLUM, 2000, VELO-ANTÓN et al. 2007, 2012, BEUKEMA, 2012, ALARCÓN-RÍOS et al. 2020).

As my results revealed, within the true salamanders, *Salamandra salamandra* has the most intraspecific variable body size, making it an excellent model species. The study of interpopulation differences of demography, reproductive parameters, and body size in the light of their complex biogeography, might be helpful to understanding interrelationships between causative factors that shape body size in true salamanders.

Acknowledgements

I thank SALVADOR CARRANZA and TONI ARRIZABALAGA for providing logistic and economic support to my project. I thank JOSE CABOT, ANNAMARIA NISTRI, JAVIER QUESADA, IVAN INEICH and DAVID DONAIRE for giving me access to the scientific collections in their care. I also want to thank two anonymous reviewers for their helpful comments.

References

- ADAMS, D. C. & J. O. CHURCH (2008): Amphibians do not follow Bergman's rule. – *Evolution*, **62**: 413–420.
- ALARCÓN-RÍOS, L., A. G. NICIEZA, A. KALIONTZOPOULOU, D. BUCKLEY & G. VELO-ANTÓN (2020): Evolutionary history and not heterochronic modifications associated with viviparity drive head shape differentiation in a reproductive polymorphic species, *Salamandra salamandra*. – *Evolutionary Biology*, **47**: 43–55.
- ALTUNIŞIK, A. (2018): Age, survivorship and life expectancy in Near Eastern fire salamander, *Salamandra infraimmaculata* (Caudata: Salamandridae). – *Russian Journal of Ecology*, **49**: 166–171.
- ANDREONE, F. & R. SINDACO (1989): Materiali per un'herpetologia del Piemonte e della Valle d'Aosta (Amphibia, Reptilia). – *Rivista Piemontese di Storia Naturale*, **10**: 205–225.
- ARNTZEN, J. W. (1995): Temporal and spatial distribution of the golden-striped salamander (*Chioglossa lusitanica*) along two mountain brooks in northern Portugal. – *Herpetological Journal*, **5**: 213–216.
- ASHTON, K. G. (2002): Do amphibians follow Bergmann's rule? – *Canadian Journal of Zoology*, **80**: 708–716.
- BAKEN, E., L. E. MELLENTHIN & D. C. ADAMS (2019): Macroevolution of desiccation-related morphology in plethodontid salamanders as inferred from a novel surface area to volume ratio estimation approach. – *Evolution*, **74**: 476–486.
- BEUKEMA, W. (2012): Homoplastic viviparity revisited: on the biogeography of salamanders in the Mediterranean Basin, with emphasis on different reproduction modes. – Doctoral Thesis. University of Twente.
- BLAIN, H.-A., J. M. LÓPEZ-GARCÍA, G. CUENCA-BESCÓS, C. ALONSO, M. VAQUERO & S. ALONSO (2009): Première mise en évidence fossile du chioglosse portugais *Chioglossa lusitanica* (Amphibia, Caudata) et son implication pour l'histoire biogéographique de l'espèce. – *Comptes Rendus Paleovol*, **8**: 693–703.
- BLOMBERG, S. P., JR. T. GARLAND & A. R. IVES (2003): Testing for phylogenetic signal in comparative data: behavioral traits are more labile. – *Evolution*, **57**: 717–745.
- BONETT, R. M. & A. L. BLAIR (2017): Evidence for complex life cycle constraints on salamander body form diversification. – *PNAS*, **114**: 9936–9941.
- BUCKLEY, D., M. ALCOBENDAS, M. GARCÍA-PARÍS & M. H. WAKE (2007): Heterochrony, cannibalism, and the evolution of viviparity in *Salamandra salamandra*. – *Evolution & Development*, **9**: 105–115.
- BUCKLEY, D., M. ALCOBENDAS & M. GARCÍA-PARÍS (2009): The evolution of viviparity in salamanders (Amphibia, Caudata): organization, variation, and the hierarchical nature of evolutionary process. – *Sociedad Española de Biología evolutiva. Adaptación y evolución, 150 años después del Origen de las Especies*: 1–6.
- DEGANI, G. (1996): *Salamandra salamandra* at the southern limit of its distribution. – Laser Pages Publishing.
- DONAIRE, D., S. BOGAERTS & D. HERBERT (2001): Confirmación de desarrollo larvario completo intrauterino en *Salamandra algira* (Bedrigo, 1883) del noroeste de Marruecos. – *Butlletí de la Societat Catalana d'Herpetologia*, **15**: 107–110.
- DOPAZO, H. J. & M. KORENBLUM (2000): Viviparity in *Salamandra salamandra* (Amphibia: Salamandridae): adaptation or exaptation? – *Herpetologica*, **56**: 144–152.
- ESTES, R. (1981): *Gymnophiona, Caudata*. Handbuch der Paläoherpetologie **2**: 1–115.
- EHL, S., M. VENCES & M. VEITH (2019): Reconstructing evolution at the community level: A case study on Mediterranean amphibians. – *Molecular Phylogenetics and Evolution*, **134**: 211–225.
- FICK, S. E. & R. J. HIJMANS (2017): WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. – *International Journal of Climatology*: DOI 10.1002/joc.5086.
- GARCÍA-PARÍS, M., M. ALCOBENDAS, D. BUCKLEY & D. B. WAKE (2003): 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.
- GRIZANTE, M. B., R. BRANDT & T. KOHLSDORF (2012): Evolution of body elongation in gymnophthalmid lizards: relationships with climate. – *PlosOne*: DOI 10.1371/journal.pone.0049772.
- HÄFELI, H. P. (1971): Zur Fortpflanzungsbiologie des Alpensalamanders (*Salamandra atra* Laur). – *Revue Suisse de Zoologie*, **78**: 235–293.
- HOUCK, L. D. & S. J. ARNOLD (2003): Courtship and mating behavior. In: SEVER, D.M. (ed.): *Reproductive biology and phylogeny of urodela* (Amphibia). – NH Science Publishers.

- KUMAR, S., G. STECHER & K. TAMURA (2016): MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. – *Molecular Biology and Evolution*, **33**: 1870–1874.
- MILLER, B. T. & J. H. LARSEN JR. (1990): Comparative kinematics of terrestrial prey capture in salamanders and newts (Amphibia: Urodela: Salamandridae). – *Journal of Experimental Zoology*, **256**: 135–153.
- ÖZETI, N. & D. B. WAKE (1969): The morphology and evolution of the tongue and associated structures in salamanders and newts (Family Salamandridae). – *Copeia*, **1969**: 91–123.
- ÖZETI, N. (1979): Reproductive biology of the salamander *Mertensiella luschani antalyana*. – *Herpetologica*, **35**: 193–197.
- PAGEL, M. (1999): Inferring the historical patterns of biological evolution. – *Nature*, **401**: 877–884.
- PARRA-OLEA, G. & D. B. WAKE (2001): Extreme morphological and ecological homoplasy in tropical salamanders. – *PNAS*, **98**: 7888–7891.
- REINHARD, S., S. RENNER & A. KUPFER (2015): Sexual dimorphism and age of Mediterranean salamanders. – *Zoology*, **118**: 19–26.
- REVELL, L. J. (2012): Phytools: An R package for phylogenetic comparative biology (and other things). – *Methods in Ecology and Evolution*, **3**: 217–223.
- RODRÍGUEZ, A., J. D. BURGON, M. LYRA, I. IRISARRI, D. BAURAIN, L. BLAUSTEIN, B. GÖÇMEN, S. KÜNZEL, B. K. MABLE, A. W. NOLTE, M. VEITH, S. STEINFARTZ, K. R. ELMER, H. PHILIPPE & M. VENCES (2017): Inferring the shallow phylogeny of true salamanders (*Salamandra*) by multiple phylogenomic approaches. – *Molecular Phylogenetics and Evolution*, **115**: 16–26.
- SALTHER, S. N. (1969): Reproductive modes and the number and sizes of ova in the Urodeles. – *The American Naturalist*, **81**: 467–490.
- SINSCH, U., H. BÖCKING, C. LESKOVAR, M. ÖZ & M. VEITH (2017): Demography and lifetime growth patterns in viviparous salamanders (genus *Lyciasalamandra*): Living underground attenuates interspecific variation. – *Zoologischer Anzeiger*, **269**: 48–56.
- TAMURA, K., F. U. BATTISTUZZI, P. BILLING-ROSS, O. MURILLO, A. FILIPSKI & S. KUMAR (2012): Estimating divergence times in large molecular phylogenies. – *PNAS*, **109**: DOI 10.1073/pnas.1213199109.
- TARKHNISHVILI, D. (1993): The ecology of the Caucasian salamander (*Mertensiella caucasica* Waga) in a local population. – *Asian Herpetological Research*, **5**: 147–185.
- VEITH, M., S. STEINFARTZ, R. ZARDOYA, A. SEITZ & A. MEYER (1998): A molecular phylogeny of the ‘true’ salamanders (family Salamandridae) and the evolution of terrestriality of reproductive modes. – *Journal of Zoological Systematic and Evolutionary Research*, **36**: 7–16.
- VELO-ANTÓN, G., M. GARCÍA-PARÍS, P. GALÁN & A. CORDERO RIVERA (2007): The evolution of viviparity in Holocene islands: ecological adaptation vs phylogenetic descent along the transition from aquatic to terrestrial environments. – *Journal of Zoological Systematic and Evolutionary Research*, **45**: 345–352.
- VELO-ANTÓN, G., K. R. ZAMUDIO, & A. CORDERO-RIVERA (2012): Genetic drift and rapid evolution of viviparity in insular fire salamanders (*Salamandra salamandra*). – *Heredity*, **108**: 410–418.
- WAKE, D. B. & N. ÖZETI (1969): Evolutionary relationships in the family Salamandridae. – *Copeia*, **1969**: 125–137.
- WAKE, M. H. (2004): Environmental effects, embryonization, and the evolution of viviparity. – in: HALL, B. K., R. D. PEARSON & G. B. MÜLLER (eds) *Environment, development, and evolution: towards a synthesis*, vol. 4. – MIT Press.
- WATT, C., S. MITCHELL & V. SALEWSKI (2010): Bergmann’s rule; a concept cluster? – *Oikos* **119**: 89–100.
- WIENS, J. J. & C. GRAHAM (2005): Niche conservatism: integrating evolution, ecology, and conservation biology. – *Annual Review of Ecology Evolution and Systematics* **36**: 519–539.
- ZHANG, P., T. J. PAPPENFUSS, M. H. WAKE, L. QU & D. B. WAKE (2008): Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudata) inferred from complete mitochondrial genomes. – *Molecular Phylogenetics and Evolution* **49**: 586–597.

Supplementary data

The following data are available online:

Supplementary document S1. Specimens used in the morphologic study of size and shape variation in terrestrial Western Palearctic salamanders.

Supplementary document S2. Bibliography used to compile data on species localities.

Supplementary document S3. Gene bank codes of the 12sRNA and cytochrome b sequences.

Supplementary document 4. Calibration points and bibliographic references used for molecular clock of the phylogenetic tree.