Ecological divergence between two evolutionary lineages of the Caucasian salamander: evidence from GIS analysis

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The Caucasian salamander (*Mertensiella caucasica sensu lato*) is an endemic taxon of the western Lesser Caucasus, classified as Vulnerable in the IUCN Red List. Two isolated evolutionary lineages occur within its range – one in the Black Sea basin, and the other in the basin of the Caspian Sea. We identified and described 51 locations throughout the range of the species, from the easternmost to the westernmost known location and within an elevational range of 50–2400 m a.s.l. We applied binary logistic regression and a maximum entropy approach to predict the range of the salamander within the polygon delimited by extreme values of 19 bioclimatic parameters for the locations identified. The models were based on the analysis of bioclimatic data, terrain ruggedness and some other environmental variables. The presence or absence of the salamander depends on the level of and variations in rainfall, temperature and terrain ruggedness. Absence of the species from the Greater Caucasus is explained by unsuitable climatic conditions. Separate analysis of the "eastern" and "western" lineages showed no overlap of their predicted ranges, and the model based on the complete data set showed good results only for the "western" evolutionary lineage. This suggests that the genetic isolation of these two lineages is caused by differential climatic requirements rather than by existing fragmentation of suitable habitats.

Key words: Caucasus, climatic preferences, *Mertensiella caucasica*, range modelling, refugia

INTRODUCTION

The Caucasus ecoregion is among the 34 worldwide biodiversity hotspots identified by Conservation International. For a non-tropical region, the Caucasus has high species diversity and endemism (Tarkhnishvili & Kikodze, 1996; Zazanashvili et al., 2004). Humid mountain forests in the west of the Caucasus have particularly high proportions of endemics (Röhrig, 1991; Tuniyev, 1990; Mai, 1995; Kikvidze & Ohsawa, 1999). This may be connected with a mild humid climate, which helped ancient biological communities to survive global climatic catastrophes (Mai, 1995; Denk et al., 2001), including the Messinian salinity crisis (Krijgsman et al., 1999) and consecutive waves of the Ice Age (Birks & Birks, 1980; Lang, 1994). It is not clear whether the entire mountain forest belt of the western Caucasus is a single continuous Tertiary refugium, or if its individual parts were isolated for long periods and should be regarded as separate refugia. There are many "pan-Caucasian" endemics, such as the wingnut (*Pterocaria pterocarpa*), the Caucasian parsley frog (*Pelodytes caucasicus*) and the long-clawed mole-vole (*Prometheomys schaposchnikowi*), but many other species are endemic to individual mountain ranges. However, the latter fact does not prove long-lasting historical fragmentation, because the ranges of many species could have been fragmented in the recent past.

The Caucasian salamander (*Mertensiella caucasica*) is found exclusively in the western part of the Lesser Caucasus Mountains, in southwest Georgia and northeast Turkey. There is evidence that the salamander is a Miocene relict. Its sister species is *Chioglossa lusitanica* from the Iberian Peninsula, and the estimated time of the separation between the ancestors of both species is about 15 million years ago (Veith et al., 1998; Weisrock et al., 2001), or even much longer (Steinfartz et al., 2007). Fossil evidence comes from Poland and Slovakia: skeleton fragments of salamanders similar to *M. caucasica* were repeatedly recorded in the Carpathian Pliocene (Sanchiz & Mlynarski, 1979; Hodrovâ, 1985). It is likely that the salamanders related to *M. caucasica sensu lato* had a wide Tertiary range, but survived the Ice Age only in the western Caucasus. Some important questions remain. The first is, why do the salamanders not occur in the Greater Caucasus, which contains habitats superficially similar to those in the areas where the salamanders do occur? Does this fact reflect a historical pattern of dispersal or, alternatively, the narrow ecological requirements of the animal and the absence of appropriate conditions in areas remote from its extant range? The second question concerns the reason for the genetic isolation of the "eastern" and "western" evolutionary lineages of the salamander (*Mertensiella sp. 1* and *M. sp. 2*), revealed by molecular genetics (Tarkhnishvili et al., 2000; Fig. 1). Are there intrinsic isolation mechanisms preventing intermixing of the lineages, or there is a geographical barrier preventing their dispersal and genetic merging? To answer these questions and to gain knowledge relevant to the conservation of the species, we developed a spatial model of the distribution of suitable habitat using GIS.

Fig. 1. Distribution of the Caucasian salamander based on locations known from the literature (test points; open circles) and those recorded in the course of the present study (solid circles). The grey polygon is defined by extreme values of 19 bioclimatic parameters (Hijmans, et al. 2005; see Appendix 2) measured at these salamander locations (BVA; see the text for details). The area of the distribution of *M. sp. 1* is delimited by an ellipse.

MATERIALS AND METHODS

Mapping the range

The literature provided information on 40 salamander locations since the description of *Mertensiella caucasica* (Tarkhnishvili & Gokhelashvili, 1999; Kaya, pers. comm.). The geographic position of most of these locations is described roughly and cannot be used for precise range modelling. For this reason, we conducted field work throughout the entire range of the salamander, from Borjomi Gorge in Georgia to the Ordu area in Turkey, during two field seasons (2006 and 2007). The field trips covered the second half of June and the first half of July, when it is relatively easy to find both adults and larvae (Tarkhnishvili & Serbinova, 1993). We recorded 26 GPS locations for salamanders in Georgia (including 12 new ones) and 25 locations in Turkey (including 14 new ones) (Appendix 1), using a Garmin Etrex 12 Channel GPS unit (Garmin Ltd, Olathe, KS, USA). Our field work covered the entire range of the salamander and a broad range of ecological conditions: the elevation of individual locations varied between 40 and over 2,400 m a.s.l., and the locations were from all types of landscape where the salamanders have ever been recorded, including mixed and broadleaf forests, and near and above the timberline.

For model validation, we used the locations known from the literature ("test points"). These are shown in Figure 1. Eleven easternmost locations mapped during the field work and four easternmost test points are from the area of distribution of *M. sp. 1*; the remaining 40 locations

mapped during the field work and 11 test points are from the area of distribution of *M. sp. 2*.

Habitat variables

In most GIS-based analyses of the distribution of amphibians, terrain, climate, and vegetation cover are used as independent variables (Arntzen, 2006; Arntzen & Teixeira, 2006). We downloaded free online data and managed them using ArcView v. 3.3 GIS software. We used bioclimatic data from WorldClim Version 1.4 (http://www.worldclim.org); this is a set of global climate layers (climate grids) with a spatial resolution of a square kilometre (Hijmans et al., 2005; Appendix 2).

Terrain data were measured from the Shuttle Radar Topography Mission (SRTM) elevation data in the UTM projection (dataset from the Global Land Cover Facility, http://www.landcover.org) at a resolution of 90 m. We measured ruggedness by calculating SD of slope within a 450 m radius, averaged from a 90 m slope grid. These data were also used to calculate potential annual direct incident radiation (megajoules/cm^2/yr) (McCune & Keon, 2002). Vegetation cover productivity was measured from 1000-m NDVI time series maps and snow cover from status maps, both provided by the VEGETATION Program (SpotImage/VITO, http://www.vgt.vito.be). We used 2001-05 VGT-S10 data for both variables.

Model development and validation

We measured highest and lowest values for each of the 19 bioclimatic variables at the 51 presence locations (Fig. 1, Appendix 2), mapped the polygons delimited by the ex-

treme values for each variable, and overlaid them to gain a single polygon delimiting extreme climates where the salamanders are found (hereafter "Bioclimatic Variables Polygon" or BVP). Then, we generated a total of 800 random points ("absence points") within those parts of the BVP from which salamanders were definitely absent according to both our data and the literature (e.g. the Greater Caucasus, the Likhi Range, central and eastern parts of the Trialeti Range in Georgia, and parts of NE Turkey west of the westernmost edge of the salamander's distribution). For habitat modelling, we used binomial logistic regression, LR (Hosmer & Lemeshow, 1989; Menard 2002). The analyses were performed in SPSS v.11 for Windows (SPSS Inc., Chicago, IL, USA) with a forward stepwise entry of independent variables. We developed the model based on the 51 presence and 700 absence points. Overlaying grids, extracting the values of individual variables and visualization of the model were conducted using ArcView GIS 3.3.

LR analysis was based on all 25 non-transformed variables, regardless of the presence of multicollinearity, using $P<0.05$ for entry and $P>0.10$ for removal (Appendix 2). We used ROC (Receiver Operated Character) curve analysis (Hanley & McNeil, 1982; Zweig & Campbell, 1993) to define 1) the strength of agreement among observed and predicted data and 2) classification cut-off values that equally balanced sensitivity and specificity. Model predictive accuracy was validated using a test presence/absence dataset based on the kappa statistic (Fielding & Bell, 1997; Scott et al., 2002). The test presence/absence dataset included 15 relatively accurate presence locations collected from the bibliography (test points), which were greater than 5 km from the 51 presence points used for development of the model (Fig. 1) and 100 absence points. Probability models were mapped within the BVP.

Because the model obtained showed highly asymmetric predictive values for *M. sp. 1* and *M. sp. 2*, we repeated the analysis separately for the two lineages, based on the same environmental variables. The maximum entropy approach (ME) was applied in this case (Phillips et al., 2006; Phillips & Dudik, 2008), a method that does not require selection of absence locations for the analysis and can operate with a low number of presence locations. The software applied was Maxent v. 3.2.19 (www.cs.princeton.edu/~schapire/maxent). To test the models, we randomly selected 25% of the original presence points. The analysis produced separate probability polygons for *M. sp. 1* and *M. sp. 2*. By multiplying these polygons, we derived probabilities for the simultaneous presence of both lineages.

RESULTS

The actual range of the Caucasian salamander

The salamanders are found between Borjomi Gorge in Georgia and Ordu District in Turkey (approximately 50 km S of Ordu). The range of the species covers the extreme north-west of the Trialeti Range, the Meskheti Range from Borjomi Gorge to the Black Sea Coast, and the northern slopes of the Doğu Karadeniz Mountains. Our field stud-
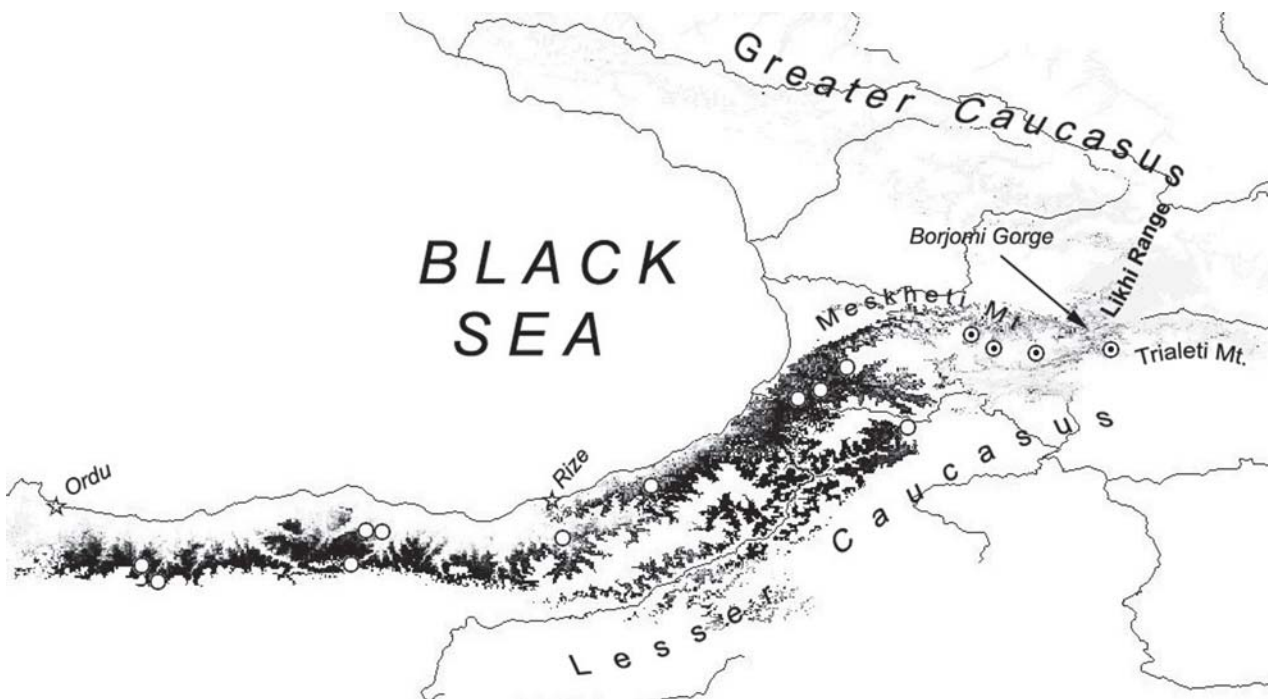

Fig. 2. Predicted range (the spatial distribution of presence probabilities) of the salamander suggested by logistic regression analysis, based on the 51 presence points that include both *M. sp. 1* and *M. sp. 2* populations. Open circles indicate 15 test points used for the model validation. Three out of four test points of the "eastern" population (circles with points) lay outside the predicted range.
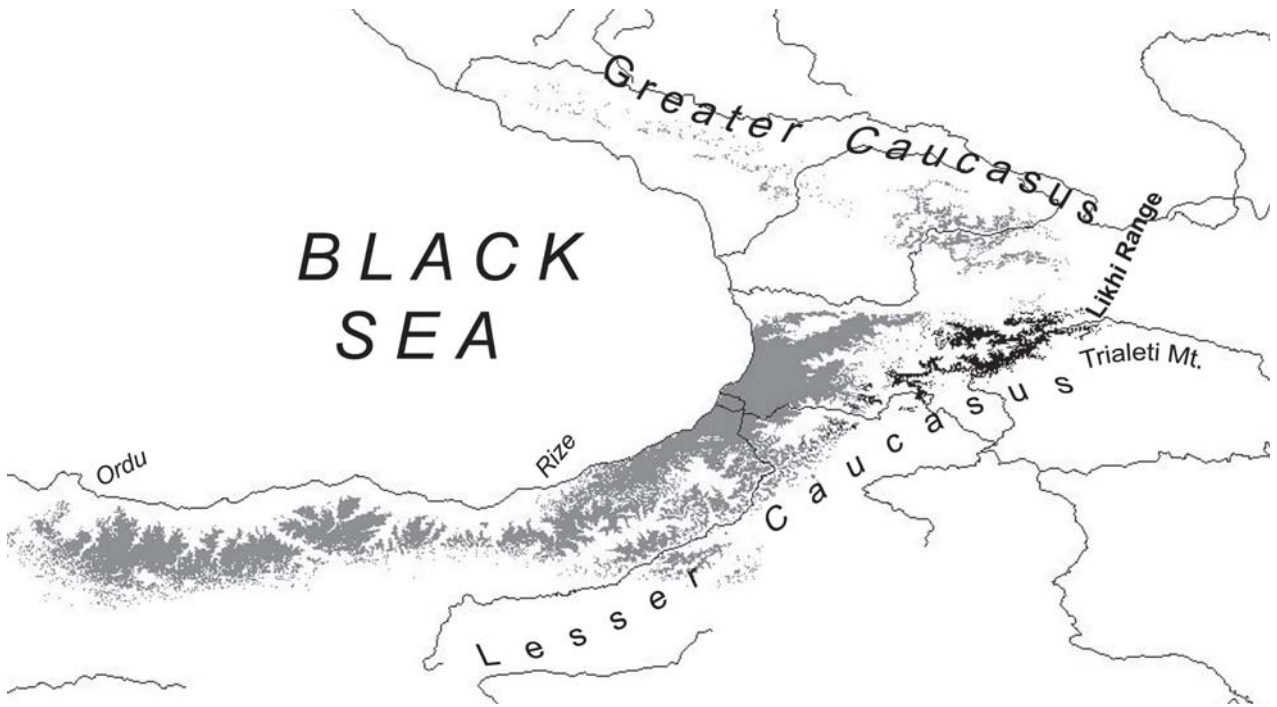
Fig. 3. Predicted range of *M. sp. 2* (grey polygon) and *M. sp. 1* (black polygon) as suggested by maximum entropy analysis, based on 40 presence points for "western" populations (*M. sp. 2*) and 11 presence points for "eastern" populations (*M. sp. 1*). Cut-off value = 0.5. Note that between the two polygons, there is a considerable area with low suitability for either lineage.

ies additionally revealed the presence of the salamander in the Çambaşı area south of Ordu, about 50 km west of the known westernmost location of the species, on the northern slopes of the Shavsheti Range in Georgia, in Camili National Park in Turkey and in the north-east of the Meskheti Range. The records of the species afrom the present study are shown in Figure 1.

Modelling the range with logistic regression

The spatial polygon delimited by the extreme values of 19 bioclimatic variables measured at the 51 presence locations (BVP) is shown in Figure 1. Bivariate stepwise logistic regression (option "Forward LR") at a cut-off value of 0.07 that equally balanced sensitivity and specificity correctly classified 91.5% of 700 absence locations and 92.2% of 51 presence locations. The model included seven variables (maximum temperature of warmest month, mean temperature of driest and warmest quarters, precipitation level during the driest, warmest and coldest quarters, and ruggedness of the terrain). Vegetation indices and variables such as snow cover and radiation index did not have a significant influence on the probability of salamanders being present. The output of the analyses is presented in Table 1. The Greater Caucasus, the Likhi Range and the eastern part of the Lesser Caucasus, where the salamanders do not occur, remained outside the predicted range. The predicted range was continuous throughout the area of the distribution of *M. sp. 2*, but strongly fragmented throughout the area of the distribution of *M. sp. 1*. It covered the area that included all but two presence locations of *M. sp. 2* but only seven out of eleven (64%) presence locations of *M. sp. 1* (Fig. 2). Validation of the model using 15 test points and 100 absence locations also showed controversial results: three out of four locations of *M. sp. 1* remained outside the predicted range (Cohen's kappa = 0.724).

Modelling ranges of *M. sp. 1* and *M. sp. 2* with the maximum entropy approach

The ME-based spatial models were separately developed for the two lineages. The spatial outputs of the models (probabilities of presence exceeding 0.5) are shown in Fig. 3. Test AUC was 0.992 for *M. sp. 2* and 1.00 for *M. sp. 1*.

The polygons show no overlap area. Multiplying probability polygons of the two spatial models produced a narrow area separating the predicted ranges of *M. sp. 1* and *M. sp. 2*, with product probabilities varying in the range 0.1–0.384.

DISCUSSION

The predictive value of spatial–environmental models of animal distribution depends on the representativeness of the areas both within and outside the species range (Arntzen, 2006; Arntzen & Teixeira, 2006). Since the presence points covered the salamander's entire range, we suggest that our models satisfactorily describe its actual distribution.

The salamanders from the easternmost part of the range (*M. sp. 1*) have fully diagnostic mitochondrial haplotypes and RAPD alleles, separating them from the salamanders throughout the rest of the range (*M. sp. 2*)

Table 1. The best-fit model for Caucasian salamander habitat, estimated using binomial logistic regression based on the 51 presence and 700 absence points.

Parameter code	Parameter estimate	S.E.	Wald	Sig.
bio_9	–0.059	0.011	27.897	0.000
bio_5	0.270	0.076	12.558	0.000
bio_19	0.040	0.010	17.711	0.000
bio_18	–0.099	0.017	34.515	0.000
bio_16	0.050	0.011	20.997	0.000
bio_10	–0.204	0.062	10.851	0.001
rug_sd	0.045	0.010	20.293	0.000
Constant	–33.122	9.719	11.614	0.001
2 Log Likelihood	133.256			
Nagelkerke R^2	0.702			
df	1			
Optimal cut-off	0.07			
AUC	0.977			0.000

(Tarkhnishvili et al., 2000). The differences revealed may reflect genetic isolation lasting 5–7 million years or even more.

The results of the binomial logistic regression analysis showed that the distribution of suitable habitats depends on a set of climatic variables and terrain ruggedness. However, LR based on the merged set of presence data for both *M. sp. 1* and *M. sp. 2* produced a spatial model with a high predictive value for *M. sp. 2* but a low one for *M. sp. 1*. *M. sp. 1* occurs in colder and drier habitats with higher temperature variations than *M. sp. 2* (Appendix 2). The separated analysis of suitable habitats for the two lineages showed quite a narrow area where the probability of simultaneous presence of the two lineages exceeds 0.1 (but never reaches 0.4). In fact, the two lineages do not overlap, not only in their actual range, but also in the predicted distributions. This pattern indicates that both lineages are limited by unfavourable climates that prevent further expansion along an environmental gradient (Costa et al., 2008); in other words, they have different requirements for temperature and humidity level.

Suitable habitats for *M. sp. 2*, as predicted by the ME model (Fig. 3), cover the northern slopes of the Doğu Karadeniz Mountains east of Ordu, and the western part of the Meskheti Range. Between Rize and Batumi, the potential range approaches the Black Sea, but east and west of this fragment suitable habitats exist only in the mountains, remote from the coastal zone. The suitable habitats in the Greater Caucasus are small, fragmented and separated from the main part of the potential range by a wide gap of unsuitable landscape. Suitable habitats for *M. sp. 1* (Fig. 3) lie in the east of the Meskheti Range and the west of the Trialeti Range. Predicted ranges for both lineages are separated by a narrow belt of habitats unsuitable for salamanders in the centre of the Meskheti Range.

Existing GIS-based models of the optimal habitat distribution of stream-dwelling salamanders (Sequeira et al.,

2001; Teixeira & Ferrand, 2001; Arntzen, 2006; Arntzen & Teixeira, 2006) and descriptive models predicting salamander occurrence (Bailey et al., 2004) are based on the study of four to eight environmental variables. In these models, at most three climatic variables were included: annual precipitation, mean annual temperature and July temperature (Arntzen & Teixeira, 2006). The initial analysis of these variables, even along with information on the vegetation and terrain, produced an unsatisfactory model for the Caucasian salamander that includes large areas where the salamanders do not occur (Tarkhnishvili, unpubl. data). Climatic restrictions that limit the distribution of the salamander are complicated and refer to a combination of bioclimatic variables, including variations in precipitation and temperature over the year. This explains the absence of the salamanders from the Greater Caucasus, where the temperature and precipitation are more variable than in the Lesser Caucasus, even when the habitats look similar. In appropriate climates, the salamanders occur in a wide spectrum of landscapes. What salamanders do need in order to survive is a humid climate and the presence of appropriate streams or brooks (a variable dependent on ruggedness) with relatively stable water flow.

Prior to the study, we hypothesized that strong and long-lasting genetic isolation between *M. sp. 1* and *M. sp. 2* is a result of the presence of a zone of dry vegetation across the Meskheti Range (Nakhutsrishvili, 1999). This climatic gap may have a long history of existence, perhaps since the Messinian Salinity Crisis, the most likely period of separation of the lineages. This is in line with the outcome of the fossil record, suggesting that modern rainfall distribution formed between seven and five million years ago (Fortelius et al., 2002).

However, the present study suggests that climatic conditions in the supposedly refugial habitats of *M. sp. 1* ("Trialeti" priority area according to Williams et al., 2006) lie outside the optimum identified for *M. sp. 2*. Operating with the baseline information on the genetic isolation between the lineages, one could speculate about the origin and evolution of *M. sp. 1*. It is likely that at one time the area of temperate rainforests, currently restricted to the south-eastern coast of the Black Sea, stretched about 150 km eastwards of its current distribution and harboured the ancestors of the salamander. Later, the Messinian Crisis shifted the range of suitable habitats westwards, although some isolated populations of the salamander might survive. Further adaptation to drier and colder climate allowed these populations to widen their spatial niche and merge into an evolutionary lineage ecologically different from the ancestral form and genetically isolated from the populations that remained in the Black Sea Basin.

Recent publications show the importance of adaptation to differential ecological conditions at the early stages of speciation of salamanders (Steinfartz et al., 2004a,b, 2007; Giordano et al., 2006). Giordano et al. (2006) showed that topography and altitude are no less important for shaping the spatial distribution of alleles in *Ambystoma macrodactylum* than geographic isolation. This is very likely the case for *Mertensiella caucasica*, when initial geographic isolation of the edge populations

triggered adaptation to the specific climates that prevented later intermixing of the lineages.

So far, no fixed morphological differences are recorded between *M. sp. 1* and *M. sp. 2* (Tarkhnishvili et al., 2000). The coloration pattern of *M. sp. 1* is more homogenous than in *M. sp. 2*. Captive *M. sp. 1* are more mobile and active than *M. sp. 2* under the same thermal conditions, which may indicate a different species-specific thermal optimum (Serbinova, unpubl. data). It is important that *M. sp. 1* is considered as a full species that needs a separate estimation of conservation status. In terms of geographic distribution, the scientific name *Mertensiella caucasica* should be maintained for this taxon (the type locality, Zekari Pass in Georgia, is from the range of *M. sp. 1*), whereas we suggest *M. sp. 2* should be assigned a different name.

In addition to Caucasian salamanders, the area with a highly specific climate delimiting the range of *M. sp. 1* supports a number of relict plants and animals separated geographically from their relatives from the Black Sea basin and Greater Caucasus (Tuniyev, 1990). It is not yet known to what extent the geographic populations of these taxa differ ecologically or genetically from the related populations existing throughout the Black Sea basin.

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APPENDIX 1

a) Locations (latitude, longitude and elevation in m) where Caucasian salamanders (either larvae or adult specimens) were recorded during field work

#	Location	Lat.	Long.	Elev.	#	Location	Lat.	Long.	Elev.
1	Kamisvake	41.90	43.53	980	27	Bayındır 1	40.68	38.17	1552
2	Kekia	41.88	43.50	1095	28	Karapınar	40.67	38.19	1921
3	Savaniskhevi	41.92	43.46	921	29	Bayındır 2	40.68	38.16	1420
4	Baniskhevi 1	41.88	43.37	895	30	Bayındır 3	40.69	38.15	1260
5	Chitakhevi	41.82	43.30	1002	31	Turnalık 1	40.68	37.95	1378
6	Kvabiskhevi 2	41.84	43.24	1400	32	Turnalık 2	40.68	37.96	1365
7	Kvabiskhevi 1	41.84	43.23	1350	33	Çambaşı 1	40.61	37.96	1633
8	Atskuri	41.76	43.17	1018	34	İmeriksa	40.68	39.54	1706
9	Abastumani brook 2	41.79	42.87	1500	35	Sümela	40.41	39.39	1271
10	Abastumani waterfall	41.79	42.85	1449	36	Coşandere 1	40.41	39.40	1287
11	Goderdzi Forest 1	41.66	42.60	1538	37	Coşandere 2	40.42	39.39	911
12	Goderdzi Forest 2	41.65	42.60	1595	38	Uzungöl 1	40.62	40.30	1132
13	Goderdzi Subalp 1	41.64	42.58	1746	39	Uzungöl 2	40.60	40.32	1133
14	Goderdzi Subalp 2	41.63	42.57	1771	40	Uzungöl 3	40.57	40.35	1365
15	Goderdzi Subalp 3 up	41.64	42.55	1886	41	Demirkapı 1	40.56	40.41	1976
16	Goderdzi Subalp 3 down	41.63	42.55	1811	42	Demirkapı 2	40.55	40.40	2180
17	Khulo 1	41.66	42.36	840	43	Demirkapı 3	40.54	40.40	2340
18	Vedzini	41.571	41.98	593	44	Karagöl 1	41.39	41.86	1493
19	Shavsheti 2	41.58	41.96	232	45	Ayder 1	40.93	41.14	1620
20	Keda 1	41.60	41.90	291	46	Ayder 2	40.92	41.15	1813
21	Keda 2	41.66	41.90	284	47	Ayder 3	40.97	41.08	1039
22	Korolistavi	41.64	41.75	218	48	Kavrun 1	40.89	41.13	2215
23	Mtirala	41.65	41.79	939	49	Çamlıhemşin 2	41.05	41.00	275
24	Mtsvane Kontskhi	41.70	41.72	67	50	Ülküköy	41.01	40.99	395
25	Charnali 1	41.56	41.61	75	51	Fındıklı 1	41.22	41.16	120
26	Charnali 2	41.55	41.61	86					

b) List of locations known from the literature (test points) used for model validation

#	Location	Lat.	Long.	#	Location	Lat.	Long.
1	Borjomi	41.82	43.49	9	İkizdere	40.82	40.57
2	Tsinubnistskali	41.80	43.10	10	Ülküköy	41.10	41.04
3	Zekari_pass	41.83	42.86	11	Maçka	40.85	39.60
4	Sairme	41.90	42.74	12	Hamsiköy	40.68	39.44
5	Kintrishi	41.73	42.08	13	Erikli-Akçaabat	40.86	39.52
6	Keda	41.56	41.82	14	Eğribel Pass	40.58	38.42
7	Makhuntseti	41.61	41.94	15	Yavuz Kemal	40.67	38.32
8	Shavshat	41.41	42.40				

APPENDIX 2

Climate variables used in the analysis

Extreme values defined by all 51 presence points of the salamander and 14 presence points of the "eastern" species (see text for further explanation). Temperature is in °C and precipitation is in mm.

Variable code	Meaning	All locations		*M. sp. 1*	
		Min	Max	Min	Max
BIO1	Annual mean temperature	39	136	52	74
BIO2	Mean diurnal range (mean of monthly [max–min] temp)	78	118	112	115
BIO3	Isothermality (BIO2/BIO7) (*100)	32	36	34	35
BIO4	Temperature seasonality (standard deviation)	5625	8059	7272	7703
BIO5	Max temperature of warmest month	210	271	220	242
BIO6	Min temperature of coldest month	–126	22	–104	–85
BIO7	Temperature annual range (BIO5–BIO6)	232	356	324	334
BIO8	Mean temperature of wettest quarter	33	135	86	127
BIO9	Mean temperature of driest quarter	–40	190	–36	–16
BIO10	Mean temperature of warmest quarter	126	208	142	169
BIO11	Mean temperature of coldest quarter	–65	65	–44	–27
BIO12	Annual precipitation	502	2244	686	788
BIO13	Precipitation of wettest month	70	288	94	106
BIO14	Precipitation of driest month	15	84	37	47
BIO15	Precipitation seasonality (coefficient of variation)	16	41	23	31
BIO16	Precipitation of wettest quarter	189	790	–248	273
BIO17	Precipitation of driest quarter	61	335	113	142
BIO18	Precipitation of warmest quarter	61	562	211	242
BIO19	Precipitation of coldest quarter	99	691	123	159